1982

Effects of dietary fat and protein on tissue cholesterol

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EFFECTS OF DIETARY FAT AND PROTEIN ON TISSUE CHOLESTEROL

Iowa State University PH.D. 1982

Effects of dietary fat and protein on tissue cholesterol

by

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A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Animal Science
Major: Nutritional Physiology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1982

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INTRODUCTION

The major cause of human death in the United States is cardiovascular disease. Epidemiological, clinical, and experimental evidence has linked the high incidence of coronary heart disease with elevated blood cholesterol (Food and Nutrition Board, 1980; Stamler, 1979). Epidemiological data, however, also show that low serum cholesterol is associated with an increased frequency of cancer (Anonymous, 1980; Beaglehole et al., 1980). Many contributing factors and preventive measures of cardiovascular disease have been investigated. No single factor, however, seems responsible for the disease and quite possibly a synergistic response occurs when more than one factor is present.

The incidence of atherosclerosis, both in humans and experimental animals is correlated with the level of blood plasma cholesterol. The cause-and-effect relationship, however, has not been clearly elucidated. The whole diet and lifestyle as well apparently are of consequence. Human and animal studies have attempted to isolate individual dietary components and assess their effects. Fat (Barrows et al., 1980; Cevallos et al., 1979; Forsythe et al., 1979; Ide et al., 1980; Ramesha et al., 1980; Tan et al., 1980), protein (Eklund and Sjöblom, 1980; Forsythe et al., 1980; Huff and Carroll, 1980; Neves et al., 1980; Roy and Schneeman, 1981; Torre et al., 1980), cholesterol (O'Brien and Reiser, 1979; Srinivasan et al., 1979; Ide et al., 1980; Ullman and Chenoweth, 1979), carbohydrate (Behall et al., 1980; Hallfrisch et al., 1981; Kritchevsky et al., 1980; Wostmann and Bruckner-Kardoss, 1980), and fiber (Jenkins

et al., 1980; Reddy et al., 1980; Strasse-Wolthuis et al., 1980) have all been found to affect blood plasma cholesterol in experimental animals and man.

It has been unequivocally demonstrated that in most circumstances dietary polyunsaturated fat lowers plasma cholesterol (Bochenek and Rodgers, 1978; Durrington et al., 1977; Grundy and Ahrens, 1970). The American Heart Association (1978), partly due to this relationship, has recommended a reduction in total fat consumption and the substitution of polyunsaturated fat for saturated fat. Despite the considerable research, there is continued confusion as to the mechanism by which polyunsaturated fats reduce plasma cholesterol concentration (Jackson et al., 1978). The hypocholesterolemia produced by polyunsaturated fat intake could result from total body cholesterol pool redistribution or from simultaneous changes in the metabolism of cholesterol, bile acids and lipoproteins. The action of polyunsaturated fats appears to be multifaceted, which explains why no single explanation has been agreed upon.

Although mortality from cardiovascular disease is known to be associated with dietary fat intake, it has recently been suggested that dietary protein is also involved and may be as important a factor as fat. In general, animal proteins have been found to produce a hypercholesterolemic response, whereas vegetable proteins have been associated with a hypocholesterolemic response (Eklund and Sjöblom, 1980; Hevia et al., 1980; Roy and Schneeman, 1981). Evidence is still lacking on which property or component of dietary protein is involved and by what mechanism vegetable protein produces the hypocholesterolemia. The source

of dietary protein (plant versus animal), however, seems to be more important than the level of the protein (Huff et al., 1977; Srinivasan et al., 1977).

One aspect of cholesterol metabolism which is not well-understood is the effect of dietary alterations. In humans there is little opportunity to have a controlled study to evaluate a number of dietary factors affecting cholesterol parameters. For this reason the present study, with adult male rats, was designed to investigate the effects of type of dietary fat and protein on serum and tissue cholesterol levels and tissue (liver and small intestine) cholesterol biosynthesis. It was hoped that this study would help to explain fluctuations observed in serum and tissue cholesterol concentrations due to these variables.

Relative rates of cholesterol biosynthesis in the liver and small intestines were determined by use of [1-^{14}C]-octanoic acid. Dietschy and McGarry (1974) showed significant dilution of the specific activity of the intracellular acetyl-CoA pool when radiolabeled acetate was used as a precursor to determine cholesterol synthesis in *in vitro* liver slice experiments. Furthermore, another limitation to the use of acetate is the presence of a partially rate-limiting step in its metabolism prior to hydroxymethylglutaryl-CoA (HMG-CoA) reductase. Radiolabeled octanoate, therefore, was used in this study to determine relative rates of cholesterol biosynthesis because it circumvents these problems (Dietschy and Brown, 1974; Dietschy and McGarry, 1974).

REVIEW OF LITERATURE

Cholesterol is a monohydric sterol found in every tissue of the animal and is implicated in the pathogenesis of atherosclerosis. In the body, it serves four basic functions: 1) it is a structural component of cell membranes, 2) it is a precursor of bile acids and their metabolites, 3) it stabilizes the structure of plasma lipoproteins, and 4) it acts as a precursor of steroid hormones and vitamin D. Cholesterol is present in large amounts in nervous tissue and brain. It is also present in significant quantities in skin, adrenals, and liver. In the blood, the total amount of cholesterol as free cholesterol and cholesterol esters is about the same as in liver, but the two forms exist in different ratios. Approximately two-thirds of the cholesterol in plasma is esterified with fatty acids whereas most of the cholesterol in the liver is in the free form. Cholesterol is transported in the blood as part of a lipoprotein complex.

Cholesterol and cholesterol esters occur in all the lipoprotein fractions but are found primarily in the low-density lipoproteins. Esterified cholesterol normally is found in the blood and in tissues (e.g., liver, adrenals, gonads, and corpus luteum) that convert cholesterol to other biologically active substances.

In the intact animal, total body cholesterol is controlled by four mechanisms: absorption, synthesis, degradation (coversion to bile acids), and excretion (neutral steroids and degradative products). Input into total body cholesterol is by dietary intake and endogenous

synthesis. Cholesterol is excreted from the body through the bile as acidic and neutral steroids and through the intestinal mucosa as neutral steroids. Cholesterol homeostasis is maintained by an intricate balance between input and output mechanisms.

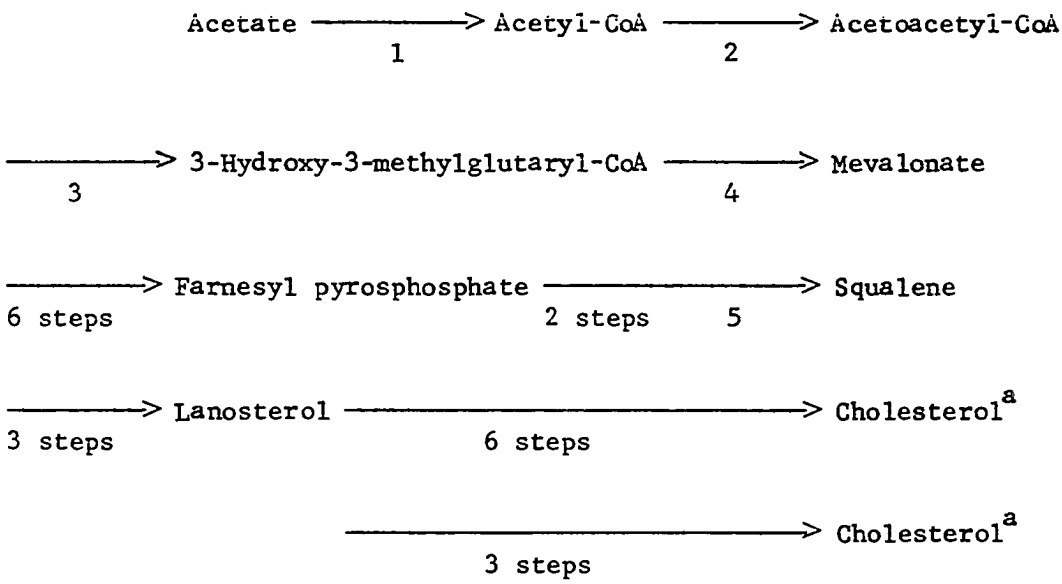
The intention of the present review of literature is three-fold: first, to review cholesterol metabolism; secondly, to discuss metabolic and dietary factors that regulate hepatic and small intestinal cholesterol biosynthesis; and thirdly, to review the effect of diet on plasma and tissue (liver and small intestine) cholesterol concentrations. The body of literature dealing with cholesterol is so extensive that only the most recent studies will be emphasized in this review.

Cholesterol Metabolism

The process of cholesterol absorption from the gastrointestinal tract was summarized by Dietschy and Wilson (1970b). Cholesterol absorbed by the small intestine originates from the diet, bile, intestinal secretion, and sloughed intestinal mucosal cells. Emulsified cholesterol esters are hydrolyzed by pancreatic hydrolase to cholesterol and free fatty acids. Essentially all cholesterol absorbed is in the free form. Cholesterol leaves the emulsion and enters mixed micelles which are present in the intestinal lumen. From the mixed micelles, cholesterol is taken up by the intestinal mucosa. Eighty to 90% of the cholesterol reaching the intestinal mucosa is taken up and reesterified by the enzyme cholesterol esterase. Cholesterol esters do not accumulate to any significant extent during active absorption, which suggests that

cholesterol ester formation is part of the terminal sequence of events in lipid absorption and seems likely to occur just prior to the exit of cholesterol from the cell (Gallo et al., 1977). The cholesterol and cholesterol ester from the lumen, along with cholesterol synthesized by the mucosal cells, then are transported by chylomicrons to the lacteals, intestinal lymphatics, and finally the thoracic lymph duct to the blood. The cholesterol components of the chylomicrons are then processed by the liver. Cholesterol absorption is therefore controlled by: 1) the physical form of dietary cholesterol; 2) the pool size of bile acids; 3) the permeability status of the lumen mucosal membrane; 4) secretion and activity of the hydrolyzing enzymes; and 5) the luminal concentrations of fatty acids, triglycerides and phospholipids derived from dietary fats (Dietschy and Wilson, 1970b).

According to present knowledge, all twenty-seven carbon atoms of cholesterol are derived from acetyl-CoA. These steps are summarized in Figure 1. Liver is the prime locus of endogenous cholesterol synthesis in the rat, with the small intestine ranking next in importance. In the rat, these organs contribute about 80% of the total endogenous cholesterol (Dietschy and Wilson, 1970b). However, there are large amounts of squalene and methyl sterols in skeletal muscle. Also, in vitro incubation of skeletal muscle has shown that the bulk of radioactivity from acetate utilized for sterol synthesis accumulated in squalene and methyl sterols. Thus, muscle (and the kidney and adipose tissue, too) can contribute to cholesterol synthesis (Miettinen, 1978). All 27 carbon atoms of cholesterol arise from the two carbons of acetate, 15 from the methyl



1 Acetyl-CoA synthetase.

2 Acetoacetyl-CoA thiolase.

3 HMG-CoA synthase.

4 HMG-CoA reductase.

5 Squalene synthetase.

[a]Digitonin precipitable sterol.

Figure 1. Pathway of cholesterol synthesis (Siperstein and Guest, 1966)

group and 12 from the carboxyl. The obligatory steps in the biosynthetic pathway include the reduction of hydroxymethylglutaryl-CoA to mevalonic acid and the cyclization of the $C_{30}H_{50}$ hydrocarbon, squalene. Although mevalonate is the product of an obligatory step in cholesterol synthesis, it is also the precursor of several nonsteroidal products (Block, 1976). Cholesterol production is related to the needs of the organism and is therefore influenced, to some extent, by nutritional, endocrine, and neurohumeral factors.

Cholesterol catabolism to the bile acids occurs primarily in the liver (Harold et al., 1955). Free cholesterol is the preferential substrate for bile acid synthesis (McGovern and Quakenbush, 1973b). The conversion of cholesterol to bile acids involves inversion of hydroxyl group at C-3, insertion of hydroxyl groups at position 7 and 12 or both, and removal of the side chain. The rate-limiting step in bile acid formation is the 7α-hydroxylation of cholesterol (Takeuchi et al., 1974). The bile acids formed are subsequently conjugated with glycine or taurine, forming the primary bile acids cholic acid and chendeoxycholic acid. In the distal intestinal tract, the primary bile acids are deconjugated by bacterial dehydroxylation at the 7 position and are either reconjugated to the secondary bile acids, deoxycholic acid or lithocholic acid, respectively, and resorbed, or excreted as acidic steroids in the feces (Dietschy and Wilson, 1970a). In the bile and blood, the bile acids circulate as amides or conjugates of taurine or glycine. The control of bile acid synthesis is under feedback inhibition by bile acids in rat, monkey and man (Dietschy and Wilson, 1970c). Cholesterol

metabolism has been discussed fully by Kritchevsky (1967).

If the newly synthesized or absorbed cholesterol is to be excreted into the feces, it can be released from the liver and transported by the lipoproteins in the blood, transferred into or exchanged with tissue cholesterol, and finally transported back to the liver for elimination or be reutilized for lipoprotein synthesis (Miettinen, 1978). Excreted cholesterol, however, leaves the body as either acidic steroids or neutral steroids in the feces. Acidic steroids are derived from bile acids, while neutral steroids, which contain cholesterol and its derivatives, are derived from bile and intestinal epithelial cells. The enzymatic conversion of cholesterol to bile acids in the liver may be the limiting factor in man's ability to excrete cholesterol from the body (Connor, 1968). The proportion of acidic to neutral steroids excreted varies with the type of diet consumed. Diets high in polyunsaturated fats (McGovern and Quakenbush, 1973a) and fiber (Howard et al., 1965; Jacobson et al., 1973; Kritchevsky et al., 1974; Kritchevsky et al., 1977) increase total cholesterol excretion, with greater excretion as acidic than neutral steroids.

Regulation of Hepatic Cholesterol Biosynthesis

Control of HMG-CoA reductase

Employing balance studies, Schönheimer and Breusch (1933) first demonstrated that cholesterol metabolism in animals is under homeostatic control. It remained, however, for the experiments of Taylor and Gould (1950) to prove conclusively that there is compensatory suppression of

cholesterol synthesis in liver of dogs ingesting a high cholesterol diet. Siperstein and Guest (1966) attempted to elucidate the mechanism by which cholesterol feeding inhibited cholesterol synthesis within the liver cell. The negative feedback reaction involved in this phenomenon constituted an extremely sensitive homeostatic regulator of cholesterol concentration. Such a process was obviously well-suited both to counteract minor variations in dietary cholesterol and to mediate the self-regulation of endogenous cholesterol synthesis. The specific site of regulation of cholesterol synthesis was found to be the conversion of 3-hydroxy-3-methylglutarate to mevalonate, namely HMG-CoA reductase which is located on membrane bound microsomes (Siperstein and Fagan, 1964). HMG-CoA reductase is known to be the rate-limiting enzyme of cholesterogenesis in animal tissues. The feedback regulation of cholesterol biosynthesis involves direct inhibition by end products (cholesterol) rather than inhibition of enzyme synthesis or genetic repression. At least two minor sites of control for hepatic cholesterol synthesis exist between mevalonate and squalene (Gould and Swyrud, 1966). Since these inhibitions are gradual and incomplete, it is likely that they are secondary to the primary inhibition of mevalonate synthesis. Hepatic cholesterol synthesis is regulated primarily through the rate-limiting enzyme HMG-CoA reductase which is controlled by circadian rhythm, hormones, bile acid concentration, fasting, and diet.

Circadian rhythm The circadian rhythm of hepatic cholesterol synthesis was first reported by Kandutsch and Saucier (1969) using incubated mouse liver slices. Back et al. (1969) used similar techniques

and confirmed the phenomenon in rats. Hamprecht et al. (1969) and Shapiro and Rodwell (1969) have reported a diurnal variation in the activity of HMG-CoA reductase, the primary rate-limiting step in the synthesis of cholesterol, which peaks at midnight and reaches a low at noon (using a normal photoperiod of 6 AM to 6 PM of light).

The existence of a circadian rhythm in the rate of hepatic cholesterol synthesis in the rat was demonstrated *in vivo* by measuring the conversion of both [1-^{14}C]-acetate and $^{3}H_2O$ to cholesterol (Edwards et al., 1972). By both methods, there was a threefold increase in the rate of hepatic cholesterol synthesis between the nadir at noon and the peak at midnight. Circadian changes in the rate of hepatic cholesterol synthesis measured *in vivo* with [1-^{14}C]-acetate were similar to changes in activity of hepatic microsomal HMG-CoA reductase. Cholesterol synthesis in the jejunum and distal ileum was also shown to exhibit the same diurnal variation *in vivo* but with a smaller amplitude (Edwards et al., 1972).

Higgins et al. (1971) found that the increased activity of HMG-CoA reductase at midnight as compared to noon was due to increased synthesis of the enzyme protein for a brief period. Rao and Ramasarma (1971) showed a threefold increase in the synthesis of cholesterol at midnight when compared to noon. The increase in hepatic HMG-CoA reductase activity, however, was about tenfold at midnight compared to noon (Shapiro and Rodwell, 1969). Other authors have found the increase in HMG-CoA reductase activity *in vitro* with incubated liver slices (Back et al., 1969) and *in vivo* (Rao and Ramasarma, 1971) was only about threefold. Thus,

the increase in the overall synthetic rate could have been less than the increase in the enzyme activity. This suggests that HMG-CoA reductase was probably not rate-limiting at midnight. The maximum activity of HMG-CoA reductase in incubated liver slices was obtained close to midnight local time in three laboratories situated around the world with a difference in longitude 50 to 150°. It appeared as though this rhythm resulted from conditioning of environmental light-darkness cycles (Higgins et al., 1971).

Effects of feeding upon the timing of the circadian rhythm were investigated by Edwards and Gould (1971). In this experiment, rats were trained to eat from 9 AM to 1 PM under normal illumination. Cholesterol synthesis peaked at 6 PM, instead of midnight, in the trained animals. Untrained rats were most active at night and consumed the most food at this time which accounted for the midnight rise in cholesterol biosynthesis. In a similar experiment, rats were given food only from 8:30 AM to 4:30 PM daily for 4 weeks, by which time they were trained to consume the required food during the daytime. In these animals, the peak activity of HMG-CoA reductase at midnight was not observed as in animals fed ad libitum. The enzyme activity during the daytime was higher than at midnight. The authors concluded that the major rise in HMG-CoA reductase activity and the subsequent increase in cholesterol synthesis in both liver and small intestine were due to food ingestion, and not light-darkness cycles (Rao and Ramasarma, 1971). The peak activity of HMG-CoA reductase apparently is about 4 to 6 hours after food consumption, and the pattern of food intake may dictate the time of the peak.

Fears and Morgan (1976a and b) could not demonstrate a circadian rhythm in cholesterol biosynthesis when $^{3}H_2O$ or [1-^{14}C]-octanoate was used as a precursor. The incorporation of $^{3}H_2O$ into cholesterol was examined in rats fed a commercial diet ad libitum both at midday and midnight (Fears and Morgan, 1976a). Rats were given $^{3}H_2O$ intraperitoneally 1 hours before being killed. Results showed no difference between midday and midnight when $^{3}H_2O$ was used to measure the total synthesis of cholesterol. The authors believed that $^{3}H_2O$ as a substrate for cholesterogenesis gave more valid results than did the use of acetate. Acetate as a substrate is of questionable physiological relevance and its incorporation into lipids may reflect the activity of acetyl-CoA synthetase rather than total rates of lipogenesis. In further studies, Fears and Morgan (1976b) measured the rates of cholesterol synthesis before and after the consumption of a meal of commercial rat diet. Male rats were trained for 3 weeks to consume their daily food intake as a meal between 10 AM and 12 AM. In each experiment, rats were maintained on a reversed light cycle with a period of darkness from 4 AM to 4 PM. Results showed that incorporation of neither $^{3}H_2O$ nor [1-^{14}C]-octanoate into the digitonin-precipitable sterols was significantly increased postprandially compared with pre-prandially. Results obtained for the small intestine were similar to those for the liver. Use of [1-^{14}C]-octanoate as a precursor is believed to give a more accurate measurement of cholesterogenesis from acetyl-CoA than is possible with acetate (Dietschy and McGarry, 1974).

Hormones In recent years, investigations have linked HMG-CoA reductase activity with hormone levels. There is evidence for a physiological control of HMG-CoA reductase by insulin (Lakshmanan et al., 1973). Activity of HMG-CoA reductase was increased two to seven times following subcutaneous administration of insulin to both normal and diabetic rats. Reductase activity began to increase after one hour, rose to a maximum in two or three hours, and declined to control levels after six hours. The response was elicited at a time during the day when the normal diurnal variation in reductase activity approached a minimum. It was also elicited when the animals did not have access to food. Decreases of reductase activity and also decreases in diurnal amplitude within one week of onset of streptozoin-induced diabetes were observed by Dugan et al. (1974) and Nepokroeff et al. (1974). The known relationship between insulin and food consumption makes physiological induction of cholesterol biosynthesis by insulin an attractive hypothesis.

Hypercholesterolemia has been associated with hypothyroidism, and hypocholesterolemia with hyperthyroidism (Bakke and Lawrence, 1964; Kritchevsky, 1964; Peters and Man, 1950; Walton et al., 1965a; Walton et al., 1965b). Lakshmanan and coworkers (1973) injected thyroxine into rats and found an increased HMG-CoA reductase activity with maximum activity at 30 hours. Thyroxine affects cholesterol biosynthesis at a step prior to mevalonate formation (Fletcher and Myant, 1958). The hypercholesterolemia of hypothyroidism may arise, in part, from an increased hepatic output of very low density lipoprotein cholesterol, and, in part, from a decreased peripheral utilization of very low density lipoprotein

and low density lipoprotein cholesterol (Keyes and Heimberg, 1979; Wahlquist et al., 1977; Walton et al., 1965b). Although hepatic cholesterogenesis is assumed to be depressed in hypothyroidism, cholesterol catabolism is depressed even further (Miettinen, 1968).

Estrogens have been shown to reduce HMG-CoA reductase activity. Sakakida et al. (1963) injected mice with serum from stilbesterol-treated chickens and noticed decreased cholesterol synthesis. Oophorectomy in rats, fed either normal or atherogenic diet, caused increases in the concentrations of cholesterol in serum, liver, and aorta (Bai and Kurup, 1979). Administration of estradiol to oophorectomized rats in both groups caused decreases in the concentrations of cholesterol in serum, liver, and aorta. The results obtained in this study are in agreement with other reports (Ence et al., 1976; Wilson et al., 1976).

Bile acids The observation that biliary diversion resulted in an increased rate of hepatic and small intestinal cholesterogenesis led to the speculation that certain components in the enterohepatic bile circulation had a controlling effect on sterol synthesis in these organs (Dietschy and Siperstein, 1965). In general, any dietary or surgical manipulation that results in depletion of enterohepatic circulation of bile acids could lead to augmented rates of cholesterogenesis in the liver. Conversely, expansion of the bile acid pool by feeding bile acids or their derivatives could lead to decreased endogenous cholesterol synthesis.

Beher and Baker (1958) first demonstrated the inhibition of hepatic cholesterol synthesis by bile salts. Grundy et al. (1966) showed that

feeding bile salts to humans resulted in decreased conversion of acetate to cholesterol. Rats receiving a polyunsaturated fat diet had an increased biliary and fecal loss of cholesterol and bile acids (Ramesha et al., 1980). The constant removal of cholesterol and bile acids from the liver activated cholesterol synthesis in these rats. Cohen et al. (1977) and Raicht et al. (1974) showed that sodium taurocholate administration at the 0.5% level increased cholesterol absorption while depressing cholesterol synthesis. However, significant amounts of deoxycholic acid, the 7α-dehydroxylation product of cholic acid, were formed. Raicht et al. (1978) carried out an experiment to determine whether the metabolic effects were due to taurocholate and/or taurodeoxycholate. Results demonstrated that a taurodeoxycholate-enriched diet led to a decreased cholesterol synthesis. Taurodeoxycholate did not elevate cholesterol absorption.

Hepatic cholesterogenesis in vitro and in vivo in the chick was depressed by taurocholate. Conversion of cholesterol to bile acids was depressed by taurocholate. Increasing the amount of bile acids circulating between liver and small intestine reduced the synthesis of cholesterol and its conversion to bile acids (Shefer et al., 1968; Shefer et al., 1970; Shefer et al., 1973). Shefer et al. (1975), in a study with primates, showed a dose-related depression of HMG-CoA reductase activity when animals were fed chenodeoxycholic acid. The authors also measured bile acid production and noted a corresponding decrease in cholic acid in the bile. By depressing HMG-CoA reductase, chenodeoxycholic acid may limit the supply of endogenous cholesterol available for the formation

of bile acids.

The mechanism by which bile acids or bile salts influence hepatic cholesterogenesis may involve HMG-CoA reductase and cholesterol absorption. Fimognari and Rodwell (1965) showed that bile salts serve as endproduct inhibitors of the enzyme, HMG-CoA reductase, by a competitive inhibition mechanism. Barth et al. (1973) also reported reduction of HMG-CoA reductase synthesis with bile salt feeding. Hamprecht et al. (1971) showed that cholic acid feeding prevented the diurnal rise of HMG-CoA reductase activity. The exact mechanism whereby bile salts depress cholesterol synthesis at the HMG-CoA reductase level is still unclear, although most authors agree that the bile acids and salts are powerful forces in regulation of cholesterol synthesis.

Fasting Hepatic cholesterogenesis can be inhibited by fasting rats for as short a time as 24 hours. The decrease in hepatic cholesterol synthesis in fasted animals is associated with: 1) decreased enzymatic activity of HMG-CoA reductase (Bucher et al., 1960); 2) decreased enzymatic synthesis of HMG-CoA reductase (Regen et al., 1966); and 3) increased cholesterol content of hepatic microsomes (Tsai and Dyer, 1973). Bucher et al. (1960) have also demonstrated a partial block between squalene and cholesterol during fasting.

Hamprecht et al. (1969) reported that rats starved for 24 hours showed a much reduced but still detectable circadian rhythm in HMG-CoA reductase activity; the maximum reached in fasted rats was less than 3% of the maximum for fed rats and the variability of the results was large. Fasting depressed liver sterol synthesis to extremely low levels as

measured *in vitro* in incubated liver slices (Tomkin and Chaikoff, 1952) or homogenates (Cayen, 1969). On the other hand, as measured *in vivo*, fasting resulted in a reduction of sterol synthesis of about 50% as measured with [^{14}C]-acetate (Gould et al., 1959; VanBruggen et al., 1952) and 40% with $^{3}H_2O$ (Gould et al., 1959). Kandutsch and Saucier (1969) reported an almost tenfold increase in the conversion of acetate to cholesterol in liver slices of mice between 8:30 AM and 8:30 PM even though food was removed at 8 AM. Mice and rats, however, appear to react very differently to fasting.

Refeeding after fasting caused a characteristic response of hepatic cholesterogenesis. When a fat-free diet was given to rats that had been fasted for two days, hepatic cholesterogenesis rapidly increased, reached its peak three days thereafter and then declined to the fasting level after five to six days (Craig et al., 1972; Slakey et al., 1972). Rats refed a fat-containing, commercial diet did not show such responses, but the activity of HMG-CoA reductase continued to increase and was restored to the level of nonstarved rats fed a fat-containing commercial diet after five to six days (Craig et al., 1972). Ide et al. (1978a) studied the time course of HMG-CoA reductase activity and cholesterogenesis in the liver of rats refed diets containing different fats at the 10% level after 48 hours of fasting. Fasting caused a profound depression of the reductase activity and sterol synthesis. In rats refed for 30 hours, the activity of HMG-CoA reductase was restored to about one-half of the level observed in prefasting rats, irrespective of the type of dietary fats.

Effect of diet

Fat Reports on the influence of saturated versus unsaturated lipids on the synthesis of cholesterol have been conflicting. Activities of HMG-CoA reductase and cholesterogenesis in rat liver have been demonstrated to be modified by the type of dietary fat (Ide et al., 1978a, b; Ide et al., 1979; Takase et al., 1977; Triscari et al., 1978).

Triscari et al. (1978) demonstrated that corn oil diets (1%, 5%, 10%, and 20%) did not have a significant effect on the conversion of $^{3}H_2O$ to cholesterol in the liver of meal-fed rats. Diets containing 10% and 20% hydrogenated soybean oil produced 143% and 243% increases, respectively, in the rate of cholesterogenesis from $^{3}H_2O$. These effects on hepatic rates of cholesterogenesis may depend on the bioavailability of fatty acids supplied by the diets. Measurement of fat absorption in the 20% fat-fed groups indicated 89% of the corn oil and only 2% of the hydrogenated soybean oil were absorbed. The lack of stimulation of cholesterogenesis from $^{3}H_2O$ following corn oil feeding is consistent with the possibility that maximal cholesterogenesis is achieved during the meal and cannot be further induced by exogenous fatty acids. The investigators found that cholesterol synthesis peaked during a three-hour meal of a 10% corn oil diet, and did not peak again during a 24-hour period. These results are similar to those reported for 1% corn oil (Sullivan et al., 1977). In a meal-fed situation where cholesterol synthesis peaked during the course of the meal, it appeared that the corn oil could not further induce the activity of HMG-CoA reductase or cholesterol synthesis.

Results of Triscari et al. (1978) are in conflict with reports on cholesterol synthesis in incubated liver slices (Hill et al., 1960), perfused liver (Goh and Heimberg, 1973), and in vivo (DuPont, 1966a) which demonstrated an increase in hepatic cholesterogenesis following treatment with unsaturated fat in ad libitum-fed rats. Dupont (1966a) demonstrated that corn oil, when compared with tallow, increased cholesterol synthesis approximately tenfold. Unsaturated fatty acids were believed to be the preferred substrate for cholesterol esterification (Dupont, 1966b). In vitro cholesterogenesis was explored in ad libitum-fed rats and found to be increased 12 hours after the intragastric administration of corn oil (Hill et al., 1960) or feeding of safflower oil (Diller and Harvey, 1964). Goh and Heimberg (1973) found an increased cholesterogenesis when liver from ad libitum-fed rats was perfused with oleic acid. Rates of cholesterogenesis in vivo were also stimulated by dietary corn oil or beef tallow (Waterman et al., 1975). Carroll (1964) demonstrated that dietary fats stimulated cholesterogenesis; unsaturated fats were more effective than saturated fats. Bortz (1967) found feeding fat decreased hepatic lipogenesis which was followed 12 hours later by increased hepatic cholesterol synthesis. It appeared, therefore, that a relatively long period of time is required to obtain increased cholesterogenesis. Goldfarb and Pitot (1972) reported that dietary corn oil stimulated the activity of HMG-CoA reductase in proportion to the amount added to a fat-free diet. In the hamster, dietary ethyl palmitate stimulated, whereas ethyl linoleate depressed, hepatic HMG-CoA reductase (Iijima and Maruyama, 1973).

Many reports indicated that there is no significant change in cholesterol synthesis due to amount of saturation of dietary fats (Grundy, 1975). Studies have reported no increased cholesterol synthesis in animals fed dietary unsaturated fats when evaluated by measuring hepatic HMG-CoA reductase (Biederdorf and Wilson, 1965; Bochenek and Rodgers, 1979; Grundy, 1975; Wilson and Siperstein, 1959). HMG-CoA reductase has been reported, however, to be simultaneously increased 80 minutes after an intravenous infusion of intestinal lipoproteins to rats, while in vitro cholesterol synthesis remained unchanged (Nervi et al., 1976). Cholesterogenesis was unchanged following liver incubation with fatty acids ranging from 8:0 to 18:2 (Nilsson et al., 1974). Hepatic synthesis in safflower oil and beef tallow fed rats was identical (Carlson et al., 1978). Bochenek and Rodgers (1979) studied endogenous cholesterol synthesis in rat liver after animals had been on various dietary programs for a period of four weeks. Endogenous cholesterol synthesis was evaluated by measuring hepatic HMG-CoA reductase, the rate limiting enzymatic step in the metabolic pathway for cholesterol synthesis. The source of neutral dietary lipids, saturated versus unsaturated, produced no change in hepatic cholesterol synthesis.

When rats were fed different fats for two to four weeks, the activity of HMG-CoA reductase was dependent on the length and the degree of unsaturation of constituent fatty acids (Ide et al., 1978a). When saturated fats with chain lengths of 12 to 18 were the dietary fat sources and were fed at the 10% level for 19 days, fats with shorter chain fatty acids caused a lower enzyme activity compared to those with longer chain

fatty acids. The activity was also regulated by the degree of unsaturation of dietary fats; the higher the unsaturation the lower the activity. Reiser et al. (1963) also contradicted the evidence that polyunsaturated fat increased cholesterol biosynthesis. These authors fed different fat types at 30% of the caloric intake and found safflower oil depressed cholesterogenesis.

One of the nutritional factors controlling the activity of HMG-CoA reductase in the liver is the amount of dietary fat. Craig et al. (1972) observed in rats a decrease in the activity of HMG-CoA reductase in response to a change in the diets from a commercial rat diet containing fat to a fat-free diet. Incorporation of [^{3}H]-acetate into hepatic digitonin precipitable sterols was elevated by diets high in fat compared to low-fat diets (Carlson et al., 1978). Although feeding fat produced an increased pool of acetyl-CoA and acetyl-CoA derivatives, no increase in cholesterol synthesis was seen until later. Bortz (1967) concluded that some mechanism other than increased acetyl-CoA pool size caused the increase in cholesterogenesis after feeding fat.

Naseem et al. (1979) investigated the effects of a high cholesterol-high fat diet on the enzymes of cholesterol metabolism during development in rats. HMG-CoA reductase in rats fed a commercial diet was significantly greater than in those fed a high cholesterol-high fat diet, suggesting an inverse relationship. A gradual decline in HMG-CoA reductase with increasing accumulation of microsomal cholesterol was observed in rats fed a high cholesterol-high fat diet.

The mechanism by which the activity of HMG-CoA reductase is

regulated by the type and amount of dietary fat remains to be elucidated. Possibly, dietary fat-dependent responses in the activity of the reductase may be mediated by the alteration of bile acid synthesis. Ramesha et al. (1980) investigated whether the hypocholesterolemic effect of polyunsaturated oils is due to inhibition of cholesterol synthesis or increased excretion of cholesterol and bile acids through the bile and feces of rats. In rats given polyunsaturated oils, biliary and fecal loss of cholesterol and bile acids far outweighed the activation of cholesterol synthesis and thereby effectively lowered the serum cholesterol levels. The effect of saturation of dietary fat on cholesterogenesis is complicated by the fact that vegetable fats, fed for their polyunsaturated fat content, contain plant sterols. The sterols have been shown to reduce hepatic cholesterogenesis by reducing HMG-CoA reductase.

Cholesterol Hepatic cholesterol synthesis was found to be under negative feedback control by the amount of dietary cholesterol intake in rats (Siperstein and Guest, 1960), squirrel monkeys (Dietschy and Wilson, 1968), and man (Siperstein, 1970). In rats, feeding cholesterol led to a feedback inhibition of 80 to 96%, while in monkeys and man, suppressions were lower, 60 to 80% and 50%, respectively (Ho and Taylor, 1970). The rate of cholesterogenesis has been found to vary inversely with the amount of cholesterol in the liver (Frantz et al., 1954; Gould et al., 1953; Shapiro and Rodwell, 1971). In the rat, the conversion of acetate to cholesterol was reduced after prolonged feeding of cholesterol at levels of 1% to 5% of the diet by weight (Dietschy and Siperstein, 1967;

Tomkin et al., 1953). When 1% cholesterol was added to the diet, hepatic cholesterol synthesis was suppressed but the degree of suppression was greater in rats consuming unsaturated as compared to saturated fats (Bochenek and Rodgers, 1978). This was associated with greater accumulation of cholesterol in livers of rats consuming unsaturated fat and a reduction in fecal neutral sterol output by this group.

The changes in the dynamic equilibrium of cholesterol after cholesterol feeding was studied using an isotopic balance method (Mathe and Chevallier, 1979). Rats were fed either a purified diet or a commercial stock diet which stimulated hepatic cholesterol biosynthesis. Some rats fed each diet were also fed up to 0.5% cholesterol. Cholesterol feeding was found to suppress cholesterol synthesis in extradigestive tissues, in particular the liver, but did not affect cholesterol synthesis in the digestive tract. The maximal decrease of cholesterol synthesis was obtained when at least 0.2% cholesterol was added to the diet. The biosynthesis of cholesterol in vivo was studied at a number of tissue sites in rats by using $^{3}H_2O$ as precursor (Fears and Umpleby, 1979). Dietary cholesterol increased the circulating concentration of cholesterol and inhibited endogenous cholesterogenesis, with concomitant increases in the concentration of esterified cholesterol in the liver and peripheral tissue.

Inhibition of hepatic cholesterogenesis occurred as early as four hours after cholesterol feeding, coinciding with increased hepatic cholesterol content. The suppression of hepatic cholesterogenesis was most apparent after 12 hours of cholesterol feeding (Sakakida et al., 1963).

Shapiro and Rodwell, 1971). The return of hepatic synthesis to normal values following discontinuance of cholesterol feeding was related to the duration of cholesterol feeding. Taylor et al. (1956) showed that the return to normal rates of hepatic cholesterogenesis required 24 hours of cholesterol deprivation if cholesterol feeding had been for 24 hours. When cholesterol intake was for 15 days, however, recovery to normal hepatic sterolgenesis required approximately two months on a cholesterol-free diet.

Effects of dietary cholesterol on hepatic HMG-CoA reductase activity and cholesterogenesis were examined in male rats refed different types and amounts of fats for three days after fasting two days (Ide et al., 1980). Reductase activity and cholesterogenesis decreased as dietary cholesterol increased and this was not influenced by the type of dietary fat. Significant depression by dietary cholesterol occurred when 0.01% to 0.05% cholesterol was fed. The maximum inhibition of cholesterogenesis when 0.5% cholesterol was fed ranged from 70% to 80%. Sterol synthesis from [1-^{14}C]-acetate was similarly depressed by dietary cholesterol but to a greater extent.

The mechanism of action whereby cholesterol inhibits HMG-CoA reductase activity is not fully understood. Siperstein (1970) proposed a lipoprotein form of cholesterol as the feedback inhibitor based on reports that showed: 1) chylomicra, when infused through the systemic circulation, reduced hepatic cholesterogenesis and 2) removal of chylomicra by thoracic fistula stimulated hepatic cholesterol synthesis. Dietary cholesterol was found to increase the concentration of cholesterol

carried both by the apoprotein β-containing lipoproteins and by a newly appearing high density lipoprotein (Mahley and Holcombe, 1977). Siperstein and Fagan (1966) suggested that feedback control of hepatic cholesterogenesis by cholesterol involves endproduct accumulation rather than mere suppression of reductase synthesis or genetic repression of reductase based on 1) the existence of feedback inhibitors, 2) the rapidity of inhibitory action, and 3) the occurrence of feedback early in the cholesterol synthetic pathway. McNamara et al. (1972), however, demonstrated that sterol inhibition of HMG-CoA reductase activity occurred primarily by a decrease in the amount of reductase. Higgins and Rudney (1973) showed evidence of both regulation of existing reductase activity and changes in the quantity of reductase protein as essential for cholesterol inhibition of HMG-CoA reductase.

Cholesterol may function indirectly by activating synthesis of reductase inhibitors. No inhibitors of reductase activity, however, were isolated from tissue homogenates of cholesterol-fed animals (Shapiro and Rodwell, 1971) or from extracts of cultured cells grown in cholesterol-rich media (Brown and Goldstein, 1974). Rodwell et al. (1976) suggested the inhibitors may be transitory, acting only until a decrease in synthesis is initiated.

Reductase activity may also be mediated through cAMP and Mg-ATP-dependent inactivation systems. The rate of cholesterol synthesis was decreased when cAMP was added to incubated liver slices or homogenates (Raskin et al., 1974). The minimum concentrations of cAMP required for an observable <u>in vitro</u> decrease in hepatic cholesterogenesis, however,

were three to four times greater than physiological concentrations. Beg et al. (1973) demonstrated that reductase in microsomes isolated from cAMP-treated homogenates had decreased activity. A decrease in HMG-CoA reductase activity was also observed when isolated liver microsomes were incubated with Mg-ATP. The mechanism of activation and inactivation of HMG-CoA reductase may be similar to that for acetyl-CoA carboxylase in which phosphorylation inactivates the enzyme and dephosphorylation activates it (Carlson and Kim, 1973). Cholesterol may regulate HMG-CoA reductase synthesis by interfering at the level of DNA transcription (Kirsten and Watson, 1974).

Protein Type and amount of protein are dietary factors believed to affect cholesterol biosynthesis; however, no study was found in the literature which documented this effect. Kenny and Fisher (1973) found that plasma and liver cholesterol concentrations were higher in rats fed low-protein than in rats fed high-protein diets. Similar rates, however, of cholesterol absorption were found at both levels of protein intake and cholesterol biosynthesis was not assessed.

Recently it has been suggested that dietary protein source is involved in the etiology of cardiovascular disease. In general, it was found that animal protein produced hypercholesterolemia, whereas vegetable proteins were associated with hypocholesterolemia (Eklund and Sjöblom, 1980; Hevia et al., 1980; Roy and Schneeman, 1981). Evidence is lacking, however, on whether the hypocholesterolemic effect of plant protein is related to a decrease in cholesterogenesis. On the contrary, Reiser et al. (1977) found that in the rat the substitution of soy

protein for casein increased the specific activity of HMG-CoA reductase.

Carbohydrate Carbohydrate type and amount is another dietary factor which influences cholesterol biosynthesis; however, results are conflicting. Feeding high carbohydrate diets to rats (Eaton and Kipnis, 1969; Shiff et al., 1971) and to man (Nestel et al., 1970) resulted in hypercholestrolemia. Preliminary experiments in rats and rabbits showed that rates of oxidation and excretion of cholesterol were decreased by dietary carbohydrate (Carroll et al., 1978a; Falconer and Carroll, 1979).

Holt et al. (1979) designed experiments to determine whether de novo liver synthesis of cholesterol was altered by feeding sucrose- or glucose-enriched diets. Since use of acetate as an in vitro substrate for estimating cholesterol synthesis has been questioned (Dietschy and McGarry, 1974), [2-^{14}C)]-acetate and $^{3}H_2O$ incorporation into cholesterol were both used to measure cholesterogenesis. Total hepatic cholesterol content was higher and cholesterol synthesis lower in animals fed the carbohydrate-enriched diets than in chow-fed rats (Holt et al., 1979). Kelly et al. (1977) demonstrated that feeding a diet containing 73% sucrose to rats increased mesenteric lymph output of triglycerides and cholesterol and it was suggested that intestinal very low density lipoprotein production was stimulated by sucrose feeding. Rat hepatic cholesterol synthesis was reduced by the intravenous administration of lymph lipoproteins, particularly large chylomicrons, but the effect of very low density lipoproteins was not clear (Nervi et al., 1975).

Five groups of six baboons each were maintained for 17 months on a

semipurified diet containing 40% carbohydrate (Kritchevsky et al., 1980). The carbohydrates fed were: fructose, sucrose, starch, glucose, and lactose. A sixth group was used as a control and was fed bread, fruit, and vegetables. Incorporation of [^{14}C]-mevalonate into liver cholesterol was highest in the sucrose- and lactose-fed baboons and lowest in the animals fed fructose. Incorporation of exogenous cholesterol (as [^{3}H]-cholesterol) into liver cholesterol was considerably lower in the glucose-fed baboons than in any of the other groups. Baboons on the test diets, however, absorbed more exogenous cholesterol (^{3}H) than controls but biosynthesis of cholesterol was not inhibited.

Role of the Small Intestine in Cholesterol Biosynthesis

Cholesterol is required by all body tissues and can be synthesized in almost every organ but it is generally agreed that the liver and intestine produce nearly all of the human body's endogenous cholesterol (Dietschy and Wilson, 1970a). Intestinal synthesis of cholesterol has been of increasing interest as it has been demonstrated that this cholesterol enters the total body pool in the rat (Lindsey and Wilson, 1965). Cholesterol synthesis occurs throughout the length of the small intestine. Several studies, however, have indicated that the terminal ileum is the major site of intestinal cholesterogenesis (Dietschy and Gamal, 1971; Dietschy and Siperstein, 1965; Shefer et al., 1972a, b). The rate of cholesterogenesis in the intestine varies with the tissue layer. The crypt cells, where mitosis is most active, have the highest rate of cholesterogenesis compared to villi and muscle cells (Wilson, 1968).

It is now recognized, however, that cholesterol can be produced by cells on the intestinal villi as well as those in the crypts (Merchant and Heller, 1977; Muroya et al., 1977).

An early investigation by Lindsey and Wilson (1965) showed cholesterol entered the lymph and circulating cholesterol pool. The intestine is of importance to circulating cholesterol levels, since it does not exhibit a feedback-repression of synthesis; hence cholesterol is produced by the small intestine even when a high cholesterol diet is fed (Gould et al., 1953; Siperstein and Guest, 1966; Wilson, 1968). The role of the intestine as a biosynthetic site of cholesterol is possibly more important in man than in other animals. The specific activity of human serum cholesterol rarely exceeds 40% of that of the fed cholesterol, indicating that approximately 60% may come from extrahepatic sources when a cholesterol-rich diet is fed (Wilson, 1968).

Regulation of Small Intestine Cholesterol Biosynthesis

The intestine is the only extrahepatic tissue in which the control mechanism of cholesterol synthesis has been studied extensively (Dietschy, 1968). Cholesterol synthesis in the gastrointestinal tract is under the control of bile salts which inhibit the conversion of 3-hydroxy-3-methylglutarate to mevalonate through a possible mechanism of enzyme repression at the genetic level rather than competitive allosteric inhibition of the enzyme. Subsequent experiments by Shefer et al. (1973) indicated that the inhibition of the enzyme was indirect, resulting from enhanced cholesterol absorption at high bile acid concentrations.

In the biosynthetic pathway for cholesterol in the intestine, HMG-CoA reductase is the regulatory step in the reaction sequence as in the liver (Rodwell et al., 1976; Sabine, 1977). Although the liver is the most predominant source of plasma cholesterol in most animals, including man, the intestine contributes a significant proportion both of total body synthesis of cholesterol and of the total cholesterol secreted into plasma (Lutton, 1976; McIntyre and Isselbacker, 1973). Moreover, there is a possibility that under some circumstances the intestine's contribution to overall synthesis and to plasma input will be significantly greater than that of the liver since cholesterol synthesis by the intestine displays several regulatory features that differ from those in the liver (Rodwell et al., 1976; Sabine, 1977). This was particularly evident when animals were fed a diet containing cholesterol, the response to this situation being virtually restricted to the depression of cholesterogenesis in the liver and not the intestines of most animals.

Small intestine cholesterogenesis, like that in liver, is influenced by bile acids, fasting, and diet. The rate limiting steps and enzymes controlling small intestine cholesterogenesis are similar to those found in hepatic synthesis.

Bile acids

There is evidence that bile salts inhibit intestinal cholesterogenesis. Bile diversion caused increased cholesterol synthesis throughout the bowel in rat (Dietschy and Siperstein, 1965) and guinea pig (Turley and West, 1976), while bile infusion produced a decreased

cholesterol formation. Biliary diversion or β-sitosterol feeding enhanced HMG-CoA reductase in rat intestinal crypt cells. Taurocholate and taurodeoxycholate, however, decreased HMG-CoA reductase activity (Shefer et al., 1973). Hatanaka et al. (1972) demonstrated that the inhibitory activity of bile salts depended upon the presence of a 3- or 7α-hydroxy group and a carboxyl group at the terminal side chain. Inhibition was abolished when the 6 or 12 position was hydroxylated. Taurine conjugates of bile acids inhibited sterol synthesis as well as did free bile acids. Glycine conjugates of bile acids, however, inhibited sterol synthesis only about half as effectively as did free bile acids (Hatanaka et al., 1972).

The mechanism of bile acid control of intestinal cholesterogenesis is not well-understood. Cholesterol synthesis at any section of the intestine is related to bile acid concentration in the lumen at the corresponding section. Bile salts were the constituents of bile which inhibited cholesterol synthesis in intact animals (Dietschy, 1968). The regulation of HMG-CoA reductase may involve repression at the genetic level rather than competitive or allosteric inhibition (Ho and Taylor, 1970). Evidence found to support this theory are: 1) a latent period was required for inhibition of intestinal cholesterogenesis after infusion of bile salts into the intact animal, 2) absence of feedback inhibition by the addition of bile salts in vivo, and 3) the sensitivity of V_{max} but not K_m of HMG-CoA reductase to bile salts.

The reciprocal relationship between lumen bile concentration and intestinal cholesterogenesis is dependent only upon the former. However,

when rats were fed tomatine, a compound which precipitates cholesterol and makes it unavailable for absorption, the rate of synthesis of cholesterol in the intestine increased, in spite of unchanged luminal bile acid turnover and level (Cayen, 1971).

Fasting

In rats, food deprivation for 48 hours did not affect intestinal cholesterogenesis (Dietschy and Siperstein, 1967). In baboons, however, an 18 to 20 hour fast stimulated cholesterol synthesis slightly, while a 48 hour fast in man reduced intestinal cholesterogenesis at the distal duodenum by 50% (Dietschy and Gamal, 1971). Cayen (1969) also showed that intestinal cholesterol synthesis in the rat was reduced by food intake. It is therefore difficult to reach conclusions about the effects of fasting on intestinal cholesterogenesis from the limited work done with the various conditions under which experiments were performed.

Dietary

Little is known about the dietary regulation of cholesterogenesis in the small bowel. Most of the limited studies on intestinal sterol synthesis and HMG-CoA reductase have used rats fed a commercial diet (Edwards et al., 1972; Heller and Shrewsbury, 1976; Merchant and Heller, 1977; Muroya et al., 1977; Shefer et al., 1972a, b; Shefer et al., 1973; Sugano et al., 1978a). In rats fed a commercial diet, the reductase activity and cholesterol synthetic rate varied strikingly along the length of the small intestine, with ileal segments exhibiting much greater activity than jejunal segments (Shefer et al., 1972a, b; Sugano

et al., 1978b). The distribution of microsomal HMG-CoA reductase in the rat small intestine was modified by the type of diets (Sugano et al., 1980). The reductase activity in rats fed a commercial diet was two times higher in the ileum than in the jejunum, while this pattern was reversed in animals fed a purified diet. Feeding a fat-containing purified diet (10% corn oil), in comparison with a fat-free diet, resulted in a significant increase in the activity of the villous reductase. In rats fed a purified diet, the synthesis of digitonin percipitable sterols from [1-^{14}C]-acetate *in vivo* was considerably higher in the proximal than in the distal portion of the small intestine. The jejunal activity for synthesizing sterols *in vitro* was also considerably higher in rats fed a purified diet than in the rats fed a commercial diet. Comparisons of cholesterogenesis of the small bowel and liver denote the possibility that intestinal contribution to endogenous cholesterol synthesis is considerably greater than previously estimated.

Andersen and Dietschy (1977) investigated the physiological regulation of sterol synthesis in a variety of tissues of rats. Although they did not give any explanation, cholesterogenesis from [1-^{14}C]-acetate *in vitro* in rats fed a synthetic diet through a gastric tube was the same in the jejunum and ileum, in contrast to a two- to fourfold higher activity in the ileum than in the jejunum in rats fed a commercial diet.

The exact mechanism causing diet-induced modulations in the distribution of intestinal reductase activity and cholesterogenesis is not known at this time. Several explanations are plausible. First, although it has been claimed that there is generally an inverse relationship between

the concentration of bile acids at any level of the intestinal lumen and the rate of cholesterol synthesis in the adjacent bowel wall (Dietschy and Wilson, 1970b), recent evidence indicates that the cholesterol content of the intestinal cells is responsible for a major rate-limiting effect upon intestinal cholesterogenesis (Sabine, 1977; Westergaard and Dietschy, 1976). Since absorption of cholesterol occurs mainly in the jejunum (Sabine, 1977), a low absorption rate in the ileum would produce higher HMG-CoA reductase activity in that portion. Sugano et al. (1980) found that HMG-CoA reductase activity and sterol synthesis are dependent upon the cholesterol to protein ratio in the villous cells. The higher the ratio, the lower the reductase activity and intestinal cholesterogenesis. In addition, the diet dependent difference in the bile flow (Balmer and Zilversmit, 1974) and the distribution of bile salts along the small intestine (Eastwood and Boyd, 1967) may directly or indirectly account for the changes in cholesterogenesis.

Nonnutritive fiber may be responsible for differences in intestinal HMG-CoA reductase activity and cholesterogenesis between rats fed commercial and purified diets. In a study conducted by Sugano et al. (1980), the HMG-CoA reductase activity in the ileum of rats fed a commercial diet was two times higher than that in the ileum of rats fed a purified diet. The commercial diet allegedly contained 4% crude fiber (manufacturer's statement), the same percentage as that of cellulose in the purified diet. However, when analyzed, the commercial diet contained 30% rather than 4% fiber. Nonnutritive fiber was postulated to be the dietary factor responsible for the differences in intestinal HMG-CoA reductase

activity and cholesterogenesis. The effect of dietary wheat bran and citrus pulp on small intestine HMG-CoA reductase activity was studied in rats (Smith-Barbaro et al., 1981). Small intestine HMG-CoA reductase was significantly lower in the wheat bran group than in either the control group or citrus pulp group. HMG-CoA reductase activity in the small intestine was 33% lower in the citrus pulp group and 55% lower in the wheat bran group than in the control group. Thus, the content of fiber may be the most plausible factor leading to different responses due to dietary manipulation.

Intestinal cholesterogenesis has been reported by some to be inhibited by dietary cholesterol (Bricker et al., 1972; Fishler-Mates et al., 1974), whereas others were not able to observe any effect (Cayen, 1969; Dietschy and Wilson, 1970c). Cholesterol synthesis in the small intestine, compared to liver, was less susceptible to feedback inhibition by dietary cholesterol. This effect was reported for several animals including rats (Dietschy, 1968), baboons (Wilson, 1968), and man (Dietschy and Gamal, 1971). In dogs (Gould et al., 1953), and monkeys (Dietschy and Wilson, 1968), dietary cholesterol decreased cholesterogenesis in small intestine only slightly or not at all. The HMG-CoA reductase activity of intestinal tissue was not suppressed by dietary cholesterol. The primary function of cholesterol synthesis in the small intestine is geared to epithelial turnover. However, during cholesterol ingestion when hepatic cholesterogenesis is suppressed, the small intestine contributes 50% to 80% of endogenous cholesterol in the rat (Chevallier and Lutton, 1973; McIntyre and Isselbacker, 1973).

Bochenek and Rodgers (1979) found that the effect of dietary cholesterol on rat intestinal HMG-CoA reductase varied depending upon whether animals received the dietary cholesterol with polyunsaturated or saturated fats. When cholesterol was fed with polyunsaturates, the enzyme activity in both the jejunum and ileum was significantly suppressed, whereas only the enzyme in the jejunum was significantly suppressed when cholesterol was given with saturated fats. A decrease in cholesterogenesis in the small intestine with polyunsaturated fat feeding has been reported by several laboratories (Carlson et al., 1978; Cayen, 1971; Chevallier and Lutton, 1973). In all these studies, depressed intestinal cholesterogenesis was attributed to increased intestinal cholesterol concentration, indicative of feedback control. More recently, it was observed that a diet high in cholesterol and taurocholate greatly reduced the intestinal reductase activity (Heller and Shrewsbury, 1976; Sugano et al., 1978b). It was concluded that dietary cholesterol has a negative feedback effect on intestinal cholesterol synthesis.

Incorporation of both [^{14}C]-acetate and $^{3}H_2O$ substrates into jejunal cholesterol was significantly greater in sucrose-fed animals than in chow-fed controls (Holt et al., 1979). Distal intestinal slices from chow- or glucose-fed animals synthesized more cholesterol than slices from the proximal intestine. In rats fed a sucrose-enriched diet, however, no difference in cholesterol synthesis was found between proximal and distal intestine when either [^{14}C]-acetate or $^{3}H_2O$ was used as substrate and the proximal-distal gradient did not occur (Holt et al., 1979). The mechanism for stimulation of jejunal cholesterol synthesis by sucrose

feeding is not entirely clear. Recently, it has been found that circulating lipoproteins alter the rate of cholesterol formation in many organs outside the liver including the jejunum (Andersen and Dietschy, 1977). Sucrose feeding alters the distribution of circulating lipoprotein cholesterol (Bar-On et al., 1976). Therefore, the change in intestinal cholesterol synthesis found on different carbohydrate diets could have resulted through altered circulating lipoproteins.

Effect of Diet on Blood Plasma and Tissue Cholesterol Levels

Fat

Plasma Many studies done over the past decade have shown that polyunsaturated fats have a hypocholesterolemic effect. This has led to the development of dietary programs for the management of some types of hyperlipidemia in an effort to prevent atherosclerosis. The American Heart Association (1978) has recommended a reduction in total consumption of fat and the substitution of polyunsaturated fat for saturated fat. Some authors believe that modification of dietary fat intake and and composition has already taken place in the last decade and is one reason why there has been a 15% decrease in deaths due to heart attack in the United States since 1970 (Jackson et al., 1978; Walker, 1977).

Eighteen-month-old Nebraska strain minipigs were fed diets containing 2% cholesterol and 20% corn oil, lard, or coconut oil for 12 to 18 months (Goldsmith and Jacobi, 1978). There was an increase of from 100% to 200% in total serum cholesterol as a result of feeding all three diets. The increase in total cholesterol was accompanied by a general increase

in esterified cholesterol. The lard diet produced the highest total serum cholesterol value. The greater development of atherosclerosis in animals fed diets containing lard or coconut oil than in animals fed diets containing corn oil was in agreement with the concept that polyunsaturated fatty acids reduce cholesterol concentrations and atherosclerosis. Forsythe et al. (1979) found that plasma total cholesterol was elevated 30% in pigs fed saturated fats compared with pigs fed polyunsaturated fat throughout a 14-week experimental period. Julius and Wiggers (1979) added support to these findings when they found that feeding purified diets containing beef tallow resulted in significantly higher plasma cholesterol concentrations than diets containing soybean oil.

Kritchevsky and Tepper (1968) and others (Spritz and Mishkel, 1969; Minick and Murphy, 1973) produced hypercholesterolemia and atherosclerotic lesions in rabbits with cholesterol-free, semisynthetic diets containing added saturated fat. Similar results have been reported in man in which an increase in the degree of saturation of dietary fat elevated plasma cholesterol independent of the amount of dietary cholesterol fed (Anderson et al., 1976; Nestel et al., 1975). In man, diets rich in saturated fatty acids tended to elevate plasma cholesterol, while diets containing unsaturated fatty acids tended to lower it (Stamler, 1978). The effect of a high cholesterol, high saturated fat diet on serum cholesterol was studied in six normolipidemic subjects (Tan et al., 1980). The diet increased total serum cholesterol (23%) by raising the cholesterol concentration in very low density lipoproteins (59%), low density

lipoproteins (13%), and high density lipoproteins (30%). Hegsted et al. (1965) and Keys et al. (1957) concluded that saturated fats have a cholesterol-raising effect of approximately twice the cholesterol-lowering effect of polyunsaturated fats.

In ruminants, however, the consuming of polyunsaturated fats seemed to increase plasma cholesterol. Calves consuming soybean oil filled milk had significantly greater amounts of cholesterol in blood plasma than did calves consuming tallow milk (Wiggers et al., 1977a). Barrows et al. (1980) fed young male Holstein calves a reconstituted milk containing 9% or 12% dried skim milk and 2% soybean oil, 2% tallow or 3.5% tallow. Plasma cholesterol concentration was greater for calves fed 2% soybean oil. In a second experiment, tallow and soybean oil fed in 2:1 combinations affected plasma cholesterol concentrations like the predominant fat fed alone. The greatest increases in plasma cholesterol concentrations occurred in calves fed soybean oil diets. No satisfactory explanation has yet been offered to account for the contradictory reductions (man) or increases (nonruminanting ruminant) in plasma cholesterol concentration often noted with feeding polyunsaturated fat diets.

Despite considerable research in this field there continues to be confusion as to the mechanism by which polyunsaturated fats reduce plasma cholesterol concentrations. Some have reported that polyunsaturated fats cause an increase in fecal excretion of neutral and acidic sterols, thus producing a secondary decrease in plasma cholesterol (Connor et al., 1969; Moore et al., 1968; Nestel et al., 1975). Others have disputed this finding and concluded that polyunsaturated fats merely effected a

redistribution of cholesterol within the body with a shift of cholesterol from the plasma to tissue pools (Biederdorf and Wilson, 1965; Bloomfield, 1964; Grundy, 1975). Grundy and Ahrens (1970) described two more possible mechanisms by which polyunsaturated fats produce a hypocholesterolemic response; these mechanisms are: 1) a decrease in cholesterol absorption and endogenous cholesterol synthesis, and 2) an alteration of lipoprotein composition and metabolism. However, after examination of sterol balance data in man, Grundy and Ahrens (1970) concluded that any change in plasma or tissue cholesterol must be due to a shift in body cholesterol, because type of dietary fat did not alter absorption, synthesis or excretion rates of cholesterol. There are several mechanisms by which the polyunsaturated fats may be acting simultaneously, which explains why no single unifying explanation has been agreed upon.

Tissue There is considerable evidence that dietary polyunsaturated fats, fed without exogenous cholesterol, increase hepatic cholesterol concentrations compared to diets containing saturated fats (Crocker et al., 1979; Hough and Bassett, 1975; Kellogg, 1974; McGovern and Quakenbush, 1973a; Rankins, 1973; Wiggers et al., 1977b). Hepatic cholesterol accumulation during linoleate consumption was primarily in the esterified form (Green et al., 1976; McGovern and Quackenbush, 1973a).

Egwin and Kummerow (1972) fed male weanling rats for 15 weeks diets containing 20% fat from either corn oil or beef tallow. Liver cholesterol level of beef tallow-fed animals exceeded those of rats fed corn oil by 50%. Liver cholesterol was significantly greater, however, for

calves fed soybean oil than for calves fed tallow (Barrows et al., 1980). Other studies have shown no effect of dietary fat source on hepatic cholesterol concentrations in rats (Bochenek and Rodgers, 1978; Carlson et al., 1978) or in nonruminating calves (Wiggers et al., 1977a). The animal model used in two of these studies employed adult rats refed following semistarvation, which may have influenced results.

Wiggers et al. (1977a) demonstrated no significant differences in tissue cholesterol concentration when different caloric levels of tallow were fed. Large differences, however, were observed when the tissue cholesterol concentrations of calves fed 2% soybean oil filled milk (Wiggers et al., 1977a) were compared with those of calves fed 3.5% soybean oil filled milk (Jacobson et al., 1974). For the soybean oil diets, an increase in calories from fat (30% to 50%) was concurrent with a marked increase in tissue cholesterol concentration, whereas for tallow diets no such increase occurred (Wiggers et al., 1977a).

As with hepatic cholesterol concentrations, responses in the intestines to dietary fat are variable. Crocker et al. (1979) demonstrated that intestinal cholesterol concentrations were similar regardless of dietary fat composition. In an early rat study, however, supplementation of a low-fat diet with 2% or 10% corn oil for one year increased cholesterol concentration in the gastrointestinal tract (Gerson et al., 1961). Wiggers et al. (1977a) demonstrated that cholesterol concentrations were higher in adipose tissue of soybean oil-fed calves as compared with tallow-fed calves. Similar to the calves, growing lambs fed a safflower oil reconstituted milk had a significant increase in muscle

cholesterol when compared with lambs consuming ewes' milk (Kirk, 1975). An accumulation of tissue cholesterol in response to polyunsaturated fat has also been observed in rats (Angel and Farkas, 1974) and calves (Jacobson et al., 1974).

Cholesterol

Serum Studies with experimental animals have consistently demonstrated the relationship between dietary cholesterol, plasma cholesterol concentrations and the development of atherosclerosis (Glueck and Connor, 1978). Connor et al. (1961b) and Mattson et al. (1972) with men and Kenealy et al. (1977) with goats demonstrated that an increase in the level of ingested cholesterol has a significant elevating effect on the plasma cholesterol level. Reiser (1978) points out, however, that statistically significant increases in plasma cholesterol may not result in physiological differences with respect to the development of atherosclerosis.

Mattson et al. (1972) fed prison inmates a cholesterol-enriched formula to determine their response to dietary cholesterol. Cholesterol-supplemented formulas resulted in a statistically significant (approximately 25%) rise in serum cholesterol. Thirty-two healthy men showed a statistically significant decrease in average weekly serum cholesterol values when subjects consumed cholesterol-free egg substitute versus two eggs per day (Ullman and Chenoweth, 1979). Exogenous cholesterol had a remarkable synergistic effect with a high carbohydrate diet in increasing the serum cholesterol (Srinivasan et al., 1978).

In another study, the effect of a high cholesterol-low fat diet on serum total cholesterol was studied in six different nonhuman primates (Srinivasan et al., 1979). Exogenous cholesterol, at 0.5% of the diet, increased the serum total cholesterol levels significantly in all species studied except chimpanzees. It was observed that there is no relationship between the basal serum total cholesterol concentrations and serum cholesterol response to dietary cholesterol (Srinivasan et al., 1979). For example, patas monkeys responded more than chimpanzees, although the initial serum total cholesterol concentration was highest in chimpanzees and lowest in patas monkeys. Even within a given species, the initial serum total cholesterol concentration was not reflective of the magnitude of cholesterol response.

The form in which cholesterol is ingested has been found to be important. Greater increases in plasma cholesterol levels have been reported in men fed cholesterol in egg yolk than in men fed a like amount of crystalline cholesterol. This phenomenon has been observed when crystalline cholesterol was fed without oil (Messinger et al., 1950), in oily solution (Cook et al., 1956), and in diets containing oil (Connor et al., 1961a; Gordon et al., 1958).

Schreibman (1975) has suggested four possible mechanisms by which increased dietary cholesterol may result in a rather small change in blood cholesterol: 1) by decreasing hepatic cholesterogenesis, 2) by causing an increased excretion of endogenously synthesized cholesterol in the bile, 3) by decreasing intestinal absorption of dietary cholesterol, or 4) by increasing uptake of cholesterol by peripheral tissue.

Tissue Fasted-refed rats accumulated cholesterol in the liver, which was almost entirely ascribed to the increase in the esterified form of cholesterol (Ide et al., 1980). The extent of liver accumulation of cholesterol was much smaller in rats refed safflower oil when the diet was free of cholesterol, but the effect of dietary cholesterol on the deposition of esterified cholesterol was much greater in this group. In addition, accumulation of cholestrol in the liver microsomal fraction was noted only when rats were refed a safflower oil diet with high levels of cholesterol. In rats fed a saturated fat diet, there was no increase in the concentration of microsomal cholesterol even at the highest level (0.5%) of cholesterol supplementation. The rise in liver cholesterol was detected only in rats fed an unsaturated fat diet. O'Brien and Reiser (1979), however, found no effect of dietary cholesterol on liver cholesterol of rats which were fed cholesterol-containing human-type diets although there was a pronounced effect when cholesterol was consumed as part of a purified diet.

Protein

Serum The elevation of serum cholesterol and development of atherosclerosis are dependent on the kind of protein in the diet and can probably be prevented by replacing animal protein with plant protein. Some sources of animal proteins, however, such as pork and egg whites have evoked similar levels of plasma cholesterol to those obtained with a diet containing plant protein (Carroll and Hamilton, 1975). Mortality from cardiovascular disease is well-known to be associated with dietary

fat intake, but it has long been suggested that the source of dietary protein is also involved and may be as important a factor as fat intake (Connor and Connor, 1972; Yudkin, 1957). Soybean protein was found to be hypocholesterolemic compared to casein and other animal proteins in rabbits fed low-fat diets without added cholesterol (Carroll, 1978). This has also been demonstrated in rats fed high-fat diets supplemented with cholesterol and bile acids (Yadav and Liener, 1977), and in chickens fed cholesterol (Kritchevsky et al., 1959). The hypercholesterolemic response was much less apparent in chickens fed diets without supplemental cholesterol (Hevia and Visek, 1979). Low-fat, casein-containing semipurified diets produced increased serum cholesterol and more extensive atherosclerotic lesions in rabbits than did low-fat isonitrogenous soy protein diets (Hamilton and Carroll, 1976; Huff et al., 1977; Kritchevsky et al., 1977). The source of dietary protein (plant versus animal) seems to be more important than the amount of the protein fed (Huff et al., 1977; Middletown et al., 1976; Srinivasan et al., 1977). Studies on human subjects further confirmed the general hypocholesterolemia associated with dietary proteins of plant origin compared to those of animal origin (Carroll et al., 1978b; Sirtori et al., 1977).

Ignatowski (1909) was the first to demonstrate that diet affected the development of atherosclerosis in rabbits and attributed the atherosclerotic lesions to the animal protein in the diet. Most of the emphasis since then has been on studying the effects of dietary fat and cholesterol on the development of atherosclerosis with relatively little attention devoted to dietary protein. Dietary protein can significantly

influence the level of plasma cholesterol in rabbits. Proteins derived from animal sources were generally found to produce a significant hypercholesterolemia when fed in a cholesterol-free, semipurified diet, whereas little or no elevation of plasma cholesterol was observed when proteins from plant sources were fed (Carroll and Hamilton, 1975; Hamilton and Carroll, 1974; Hamilton and Carroll, 1976). Hamilton and Carroll (1976) demonstrated the influence of different sources of dietary protein on serum cholesterol of rabbits consuming low-fat, low-cholesterol diets during a four-week feeding period. Among the animal protein sources investigated, nonfat milk had a significantly greater effect than beef protein on average serum cholesterol, 230 and 160 mg/dl, respectively. Eklund and Sjöblom (1980) found that casein and milk protein diets resulted in comparatively high levels of lower density lipoproteins, triglycerides and cholesterol, whereas low levels of these serum components were obtained with a soybean meal diet.

Kritchevsky et al. (1978) demonstrated that soy protein was less atherogenic than casein for rabbits fed a semipurified, cholesterol-free diet. The authors hypothesized that the difference between these proteins may be due to their ratios of arginine/lysine (0.49 in casein; 1.18 in soy). The authors, therefore, compared diets in which the protein component (25%) was casein, soy protein, casein plus arginine and soy plus lysine. After eight months on the respective diets, serum cholesterol was highest in the casein diet (174 mg/dl) and lowest in the soy protein (59 mg/dl) diet. The serum cholesterol in the casein plus arginine group (129 mg/dl) was higher than the soy plus lysine group

(100 mg/dl). The data show that the atherogenicity of semipurified diets can be affected by the addition of specific amino acids. Addition of lysine to soy protein diet increased atherogenicity by about 70%. Addition of arginine to a casein diet reduced atherogenicity by 26%. Conversely, Hevia et al. (1980) found that hypercholesterolemia with casein feeding was unrelated to the high lysine content of this protein.

Evidence is lacking on which property or component of the dietary protein source is responsible for the differential effects on blood plasma cholesterol. Feeding enzymatic digests or mixtures of amino acids to rabbits indicated that the difference between animal and plant protein is at least partly due to the differing amino acid composition of the proteins (Carroll, 1978). Amino acid mixtures corresponding to casein protein when fed to rabbits in a low-fat, cholesterol-free semipurified diet, produced the same degree of hypercholesterolemia as the protein itself (Huff and Carroll, 1980). Amino acid mixtures corresponding to soy protein isolate gave low plasma cholesterol levels, but not as low as those obtained with the intact protein. In further experiments, protein components were formulated by adding amino acids to casein to give a mixture corresponding to soy protein, or by adding amino acids to soy protein to give a mixture equivalent to casein. These diets failed to reverse plasma cholesterol levels, which suggests differences in digestion and absorption of proteins relative to amino acid mixtures (Huff and Carroll, 1980). Potter et al. (1979) suggested that the hypocholesterolemic action of soy protein or protein hydrolysates is attributable, not to the amino acid composition, but the presence of saponins.

Recent experiments conducted by Gibney et al. (1979), however, indicated that the hypocholesterolemic action of soybean protein is not dependent on soya saponins. Sautier et al. (1979) added further support when they found that the effect of soy protein cannot be attributed to its saponin content.

Roy and Schneeman (1981) conducted a study to determine if vegetable proteins lowered plasma cholesterol because of lower digestibility and trypsin inhibitor content of plant proteins. Mice were fed cholesterolemic diets containing either casein or soy protein isolate for four weeks. Results showed that the soy group had lower plasma cholesterol levels and increased bile acid secretion, leading to increased cholesterol catabolism. Trypsin inhibitor addition to the casein diet had no effect on cholesterol parameters.

A high dietary protein level decreased plasma cholesterol levels in rats (Nath et al., 1958) and birds (Leveille et al., 1962) receiving exogenous cholesterol. In contrast to this, Kato et al. (1978) found that the increase in plasma cholesterol due to dietary addition of 1,1,1-trichloro-2,2-bis (p-chlorphenyl) ethane (DDT), butylated hydroxy toluene, or polychlorinated biphenyls (PCB) was enhanced with a high protein diet. Hevia et al. (1979) found that rats fed 15% soybean protein with 1% cholesterol had fatty livers which were prevented with 30% and 45% soybean protein. Fifteen percent casein with 1% cholesterol did not cause fatty livers but fatty livers developed with 30% or 45% casein. Rats fed soybean protein had 12% less serum cholesterol than casein fed rats. An investigation was initiated by Torre et al. (1980) to determine

if the addition of an incomplete protein to a complete rat ration would bring about an increase in serum cholesterol. Gelatin was chosen as the incomplete protein because it is deficient in tryptophan, an essential amino acid. Adult male Sprague-Dawley rats fed rations with added gelatin at 5%, 15%, and 25% of the total ration (and substituted for other protein in the diet) for 30 days showed significant increases in serum cholesterol levels. The data indicated that hypercholesterolemia resulted from feeding the imbalanced protein. Torre et al. (1980) reasoned that the addition of an incomplete protein resulted in a larger quantity of systemic amino acids not used for protein synthesis being converted to lipids by intermediary metabolic processes.

Neves et al. (1980) studied the effect of various protein sources on fasting blood lipids in the rat. The purified proteins included casein, egg albumin, lactalbumin, soy, and alfalfa. Crude proteins studied included yeast, fish meal, and blood meal. Results showed that the pure and crude plant proteins did not have a hypocholesterolemic effect compared with pure and crude animal proteins in rats. The authors suggested that the hypocholesterolemic effects of plant proteins reported in the literature may well be limited to the specific experimental conditions employed. For example, plant proteins were hypocholesterolemic only in rats already made hypercholesterolemic with cholesterol-rich and saturated fatty acid-rich diets (Carroll et al., 1979; Yadav and Liener, 1977). The hypocholesterolemic effect of plant proteins seems to be directly related to the degree of hypercholesterolemia and physiological state (Roy and Schneeman, 1981; Sirtori et al., 1977).

Kim et al. (1978) fed pigs high fat (40% of energy, predominantly from butter), high cholesterol (1,055 mg/pig daily) diets containing either soy protein or casein for six weeks. Plasma cholesterol levels were approximately twice as high in pigs consuming casein as in pigs consuming soy protein. Julius and Wiggers (1979) found there was a general trend toward higher plasma cholesterol concentrations in pigs fed purified diets containing casein than in pigs fed purified diets containing soy protein isolate. Furthermore, Forsythe et al. (1979) found that animal protein diets (casein and lactalbumin) increased total cholesterol 23% over vegetable protein diets (soybean, corn, and wheat), but only after the pigs were on the respective diets for eight weeks. Pigs fed casein did not grow as well as pigs fed soy protein and this could, as Kim et al. (1978) pointed out, account for some of the differences in plasma cholesterol levels between treatments.

The effect of dietary protein on serum total cholesterol concentration was studied in six different nonhuman primates (Srinivasan et al., 1979). Levels of dietary protein at 6%, 12%, and 37% of calories had no main effect on serum total cholesterol in nonhuman primates. The addition of cholesterol to an 8% protein diet resulted in relatively lower serum total cholesterol than addition to a 25% protein diet. In contrast, further reduction in dietary protein to the 4% level markedly increased the serum cholesterol.

Yerushalmy and Hilleboe (1957) and Yudkin (1957) called attention to the strong positive correlation between the incidence of coronary heart disease and the amount of animal protein ingested in different countries.

There is also general agreement that severe deficiency of dietary protein is associated with a reduction of serum cholesterol (Truswell, 1975; Truswell and Hanson, 1969) and that diets high in protein have a tendency to elevate the level of serum cholesterol (Rickman et al., 1974). Anderson et al. (1971) observed no differences, however, between subjects on two different diets in which half the daily protein intake consisted of either egg white or wheat gluten. Other studies, though, have provided evidence that a change from animal protein to plant protein in the diet is associated with a decrease in the level of serum cholesterol (Hodges et al., 1967; Olson et al., 1958; Sirtori et al., 1977; Walker et al., 1960).

Several of the studies referred to above provide evidence that replacement of animal protein in the diet by plant protein is accompanied by a decrease in the level of blood cholesterol. Studies on a group of vegetarians living in the United States likewise indicated that they had lower plasma cholesterol levels than the population as a whole (Sacks et al., 1975). The effect of dietary protein on the level of plasma cholesterol in young, healthy, normolipidemic women was investigated by feeding either a conventional diet containing mixed protein or a plant protein diet in which the animal protein of the first diet was replaced by soy protein meat analogues and soy milk (Carroll et al., 1978b). The diets were fed for 78 days. In this study, the mean plasma cholesterol level was found to be significantly lower in subjects fed the plant protein diet. These findings provided an indication that the level of plasma cholesterol in humans may be influenced by the kind of dietary protein as well as

the amount in the diet.

There is good evidence that hypercholesterolemia can be reduced in human subjects by a decrease in the fat content of the diet or by substitution of polyunsaturated fat for saturated fat, but there is need for alternative methods to achieve even greater reductions in cholesterol levels, particularly for individuals at high risk from cardiovascular disease. More consideration should be given to the possibility that this can be achieved by varying nonlipid components of the diet such as protein.

Tissue Protein is another dietary constituent which seems to influence hepatic cholesterol levels. Mokady (1970) tried to evaluate the effects of protein quality on hepatic cholesterol. Male and female weanling rats were fed diets containing 10% of either casein or wheat gluten. With casein, hepatic cholesterol was 1.7 mg/g liver for both sexes. The level rose to 2.3 and 2.4 mg/g liver for males and females, respectively, when wheat gluten was fed. Thus, casein, the protein with an amino acid pattern favorable for growth, produced lower levels of hepatic cholesterol than did the imbalanced protein, wheat gluten. Hevia et al. (1980) found that increasing dietary casein from 7.5 to 15% increased plasma cholesterol and decreased liver cholesterol concentration, suggesting a redistribution of total body cholesterol from the liver to the blood plasma.

Forsythe et al. (1980) used young male pigs to examine the effects of dietary protein and fat sources on cholesterol parameters. Diets provided 16% and 42% of metabolizable energy from protein and fat,

respectively, and were fed for 12 to 14 weeks. Cholesterol content of the four experimental diets (plant protein-polyunsaturated fat; plant protein-saturated fat; animal protein-polyunsaturated fat; and animal protein-saturated fat) was 0.6 mg/kcal. Neither of the experimental variables, fat or protein, caused any change in liver or aorta lipid content. Kritchevsky et al. (1978) also found that liver cholesterol concentrations were comparable when rabbits were fed a semipurified cholesterol-free diet containing either soy protein or casein.

Carbohydrate

Serum Interest has grown recently in determining whether dietary carbohydrates, the nature and consumption of which vary widely among populations, influence atherogenesis through changes in serum lipids. Dietary carbohydrate may affect serum cholesterol concentration (Ahrens et al., 1968; Armstrong et al., 1976; Wells and Anderson, 1959). In recent years, increased consumption of sugar has been implicated in cardiovascular disease. The kind of carbohydrate consumed has been reported to affect serum lipids and the effects were greater for sucrose than other sugars or starch. The significance of Yudkin's (1966) observations relating mortality from coronary heart disease to the consumption of refined sugars has been considered by many to be questionable (McGandy et al., 1967; Paul et al., 1968).

Feeding a sucrose-saturated fat diet for six weeks produced a consistent increase in serum cholesterol in both squirrel and spider monkeys (Srinivasan et al., 1978). Polyunsaturated fat reduced the hypercholesterolemic effect of sucrose. Dextrin diets resulted in lower serum

cholesterol responses than sucrose diets when the diets contained 0 or 0.1 mg/kcal added cholesterol. Furthermore, rats fed dextrin had approximately 12% less serum cholesterol than rats fed sucrose (Hevia et al., 1979). Increasing the concentration of casein in the dextrin diet did not change serum cholesterol but increasing the concentration of soybean protein with dextrin was slightly hypocholesterolemic. Recently, Srinivasan et al. (1979) demonstrated that following six weeks of a high-sucrose diet without exogenous cholesterol, serum total cholesterol levels increased in both squirrel and spider monkeys. The addition of 0.36% exogenous cholesterol to the sucrose diet resulted in increased serum total cholesterol levels in both species. Conversely, Hallfrisch et al. (1981) fed rats ad libitum a 40% fat diet containing either 30% sucrose or 30% starch by weight for nine weeks. Results showed that cholesterol levels were not different between these two diets. Behall et al. (1980) compared the effect of sucrose and wheat starch on serum cholesterol levels of two groups of six young women. These authors found that plasma cholesterol was not significantly different after consumption of the two carbohydrate diets.

Simko (1980) demonstrated that the elevation in plasma cholesterol when sucrose is fed (47% of total calories) is related to the fructose part of the molecule. Therefore, the absence of a lipid-elevating effect when glucose is fed instead of sucrose may be of practical importance for preventive dietary programs, therapeutic nutrition, and the food industry.

The extensive use of dairy products in developed countries has been linked to the high rate of cardiovascular disease, which has focused

attention on the potential influence of lactose (Wostmann and Bruckner-Kardoss, 1980). Human consumption of lactose, however, seldom surpasses 10% of total calorie intake. At this amount, no major effects on sterol metabolism have been reported in animals or man (Wostmann et al., 1976).

Five groups of six baboons each were maintained for 17 months on a semipurified diet containing 40% carbohydrate (Kritchevsky et al., 1980). The carbohydrates fed were: fructose, sucrose, starch, glucose and lactose. A sixth group was used as a control and was fed bread, fruit, and vegetables. Serum cholesterol concentrations of the baboons fed lactose were signficantly higher than those of the fructose- or sucrose-fed animals. The terminal cholesterol concentration of the control group was signficantly lower than those of the test groups.

Wostmann and Bruckner-Kardoss (1980) maintained rats and gerbils on a casein-starch diet containing 0.1% cholesterol. Addition of 10% lactose (replacing a similar amount of starch) had no effect on serum cholesterol. Both lactose-fed and control animals showed steadily increasing serum cholesterol concentrations until 21 months when a plateau of 170 mg/dl was reached. Gerbils showed a maximum serum cholesterol concentration at 12 months of age. Serum cholesterol concentrations were higher at all times in the lactose-fed gerbils, but the serum cholesterol levels decreased during the second year of life. The data indicated that in the gerbil lactose intake in the range of possible human consumption does affect cholesterol parameters.

Tissue Kritchevsky et al. (1980) maintained five groups of six baboons each for 17 months on a semipurified diet containing 40%

carbohydrate. The carbohydrates fed were: fructose, sucrose, starch, glucose and lactose. A sixth group was used as a control and was fed bread, fruit, and vegetables. Liver total cholesterol was highest in the baboons fed lactose and fructose. The ratio of free to esterified cholesterol, a value which may serve as an indicator of cholesterol deposition, was lowest in baboons fed sucrose or glucose.

Kritchevsky et al. (1979) found that cholesterol (0.1%) fed with very high levels of lactose (40% of diet; 41.5% of calories) led to atherosclerosis in baboons. Wostmann and Bruckner-Kardoss (1980) demonstrated that the addition of 10% lactose to a casein-starch diet containing 0.1% cholesterol had no effect on aortic cholesterol in the rat. In gerbils, however, the addition of 10% lactose significantly increased aortic cholesterol content.

Fiber

Serum Epidemiological studies (Burkitt et al., 1974) have suggested that the development of atherosclerosis in the western world is linked to a low intake of dietary fiber. The addition of fiber to the diet, however, does not consistently produce hypocholesterolemia. The nutritional importance of the nondigestible component of the diet is now recognized and is defined as substances resistant to digestive enzymes secreted by mammals (O'Brien and Reiser, 1979). Some of these nondigestible diet constituents are hypocholesterolemic (Kay and Truswell, 1977b; Tsai et al., 1976) as are plant sterols (O'Brien et al., 1977).

A number of epidemiological studies suggested a relationship between high fiber intake and decreased incidence of coronary heart

disease (Cummings, 1973; Trowell, 1975; Trowell et al., 1974). Dietary studies on vegetarians indicated that vegetable diets lower blood cholesterol (Hardinge et al., 1958; Hardinge and Stare, 1954; Ruys and Hickie, 1976). The results were presumably due to vegetable fibers. Jenkins et al. (1980) found that when eleven hyperlipidemic patients took an average of 13 g guar in crispbread form over two- to eight-week periods, a reduction of total serum cholesterol of 13% resulted. Strasse-Wolthuis et al. (1980) studied the effects of dietary fiber from different sources on cholesterol parameters in a group of 62 healthy volunteers under strict dietary control. Results showed that the concentration of serum total cholesterol decreased in those subjects on diets containing either citrus pectin or vegetables and fruits, by 13 and 7 mg/dl, respectively. The addition of bran, however, caused a statistically significant increase in serum cholesterol of 13 mg/dl. Plasma cholesterol levels were reduced in rats fed 15% pectin or carrageenan in the diet, but were not altered by the addition of wheat bran or alfalfa to the diet (Reddy et al., 1980).

Rabbits developed hypercholesterolemia and atherosclerosis when fed cholesterol-free, semipurified diets but not when fed commercially formulated diets of natural ingredients (Hamilton and Carroll, 1976). Since commercial diets frequently contain considerably more fiber than semipurified diets, fibrous material from various sources were added to a semipurified diet to see whether hypercholesterolemia could be prevented. The results were negative; in fact, the addition of large amounts of powdered cellulose actually seemed to enhance the hypercholesterolemia.

Rabbits on a commercial diet had a faster rate of cholesterol turnover than rabbits fed a low-fat, cholesterol-free, semipurified diet. This difference seemed to be more related to rates of cholesterol oxidation than to rates of fecal neutral sterol excretion. Similar differences in rates of cholesterol oxidation were observed between rats on semipurified and commercial diets (Hamilton and Carroll, 1976).

Several possible mechanisms exist for the ability of certain fibers to lower plasma cholesterol. Eastwood and Boyd (1967) found that significant quantities of bile acids were bound to nonabsorbable materials in the small intestine. Increased excretion of fecal sterols and bile acids was observed in rats fed an atherogenic diet when the fiber intake was high (Menon and Kurup, 1976). In further experiments (Eastwood and Hamilton, 1968), it was found that this binding of bile salts by dietary material could be duplicated in vitro and that lignin, a constituent of most types of fiber, actively bound bile salts from phosphate buffer solutions. The results obtained by Story and Kritchevsky (1976) indicate that lignin binds various bile acids. The information suggested that fiber decreased serum cholesterol by binding bile salts. Such binding could result in a failure in micellar formation, essential for cholesterol absorption, and this, in turn, would increase bile acid excretion, resulting in an increased bile acid synthesis to replace the lost bile salts. Both events would drain cholesterol pools. Story et al. (1977) suggested that a dietary level of fiber higher than 10% may be required to produce a consistent effect on cholesterol metabolism in the rat, a species which is very efficient in adjusting its rate of

cholesterol synthesis and degradation in response to dietary intake of cholesterol. While studying the influence of dietary alfalfa, bran, and cellulose on cholesterol metabolism in the rat, these authors found that the serum cholesterol levels were not consistently lowered by 10% fiber. Changes in the enzymes involved in cholesterol synthesis and degradation were observed but these did not exhibit a trend in any particular direction.

Bile acids are the main products of cholesterol oxidation, and reports indicate that more bile acids are produced by commercial than by semipurified diets, both in rats (Grundy et al., 1965; Portman, 1960) and in rabbits (Grundy et al., 1965; Hellström et al., 1962). The higher rate of production of bile acids by animals on commercial diets might be a compensatory response to increased loss of bile acids and bile salts in the feces. This in turn could be due, as discussed above, to binding of bile salts and bile acids to indigestible fibrous material in the diet. Story and Kritchevsky (1976) found that the binding affinity of fiber for bile salts and bile acids depends on the type of fiber and also on the bile salt and bile acid involved.

Kritchevsky and Story (1975) have pointed out the shortcomings of equating the binding capacity of certain fibers for sodium taurocholate with their ability to bind all bile acids. The authors compared the binding capacity of several types of fiber for the major bile acids and their conjugates. Lignin seems to have a greater overall capacity for binding than any other type of fiber. Cellulose seems unable to bind any bile acid, indicating its lack of effect on lipid metabolism in the

intact animal. Bran binds relatively little taurocholate but binds appreciably greater amounts of other bile salts. Alfalfa also binds less taurocholate than any of the other bile salts tested.

Despite the fact that bran has some capacity for binding bile acids and bile salts in vitro (Story and Kritchevsky, 1976), many studies with humans (Baird et al., 1977; Kay and Truswell, 1977a, b; Truswell and Kay, 1976) and rats (Kay and Truswell, 1975; Truswell and Kay, 1975) failed to show that wheat fiber lowered serum cholesterol or increased fecal sterol excretion. Arvanitakis et al. (1977) observed that incorporation of wheat bran, which is rich in fiber, at the 10% level did not affect serum cholesterol and triglycerides in rats fed control or atherogenic diets. However, the substitution of white flour for whole wheat flour in the diet elevated the serum cholesterol level in a community of Cistercian monks who were all lactovegetarians (Eastwood, 1969). Rats that received a bran diet as compared with a no-bran diet showed increased serum cholesterol concentration (Forsythe et al., 1977).

The effect of administering blackgram (Phaseolus mungo) fiber at the 30% dietary level was studied with regard to the concentration of biliary and fecal bile acids (Jayakumari and Kurup, 1979). The results indicated that blackgram fiber significantly lowers serum cholesterol in rats. There was an increased concentration of biliary sterols and bile acids and increased fecal excretion of sterol and bile acids, each of these effects being significantly greater than those observed in rats fed cellulose. The decrease in serum cholesterol was manifested both in the β- and pre-β-lipoproteins as well as in the α-lipoproteins. Thus,

it is clear that the cholesterol-lowering action of dietary fiber is not due to cellulose alone.

Carob pod fiber containing 44.4% highly polymerized tannins was fed to rats in a purified diet to study its effect upon the concentration of plasma cholesterol and the fecal excretion of sterols and bile acids (Wursch, 1979). Results indicated that carob fiber had an influence on cholesterol turnover by decreasing the absorption of bile acids and cholesterol but the latter only in the case of cholesterol-fed rats.

Many fiber-containing materials possess, in addition to their bile salt-binding capacity, physical characteristics that could influence lipid metabolism. Early reports stated that vegetable fiber increases the amount of the volatile fatty acid in the feces (Williams and Olmsted, 1936), increases the fecal weight, and shortens the transit time (Cummings et al., 1978). Eastwood (1977) suggested that large fecal output may be affecting cholesterol metabolism. The water-holding capacity of fiber, which results in a great increase in total fecal output, could influence the absorption of cholesterol and bile acids. McConnell et al. (1974) measured the water-holding and ion-exchange capacities of many fiber-containing materials, including turnip, carrot, celery, and apple. These authors theorized that in the large intestine these materials may act as a physiological chromatography column, whose gel-filtration and ion-exchange properties may influence bile acid absorption and, hence, cholesterol metabolism.

Tissue There are not many studies in the literature that discuss the effects of fiber on tissue cholesterol concentrations and the ones

available are not in agreement. Chang and Johnson (1980) demonstrated that pectin decreased liver cholesterol concentration in rats fed a 12% corn oil or lard diet. Chang and Johnson (1978) demonstrated that animals fed cabbage had lower liver cholesterol concentrations with sucrose and cornstarch diets than with a white wheat flour diet. Dry feed supplementation (high fiber) decreased total body cholesterol concentrations of calves fed either soybean oil or tallow (Barrows et al., 1980). However, Reddy et al. (1980) found liver cholesterol levels comparable in rats fed pectin, carrageenan, wheat bran, or alfalfa as a source of fiber. Arvanitakis et al. (1977) observed that incorporation of wheat bran, which is rich in fiber, at the 10% level did not affect liver cholesterol in rats fed control or atherogenic diets. Likewise, Story et al. (1977) found that liver cholesterol levels were not consistently lowered by 10% fiber. The mechanism whereby fiber alters tissue cholesterol concentrations has not been established.

MATERIALS AND METHODS

Experimental Design

The experiment was designed to study the effect of type of dietary fat and protein on plasma and tissue cholesterol levels and tissue (liver and small intestine) cholesterol biosynthesis in adult male rats. Tables 1 and 2 diagram the dietary treatments and sacrifice schedule used.

Table 1. Dietary treatments

Dietary group	Dietary treatments	
	Pretreatment (Weaning to day 0)	Experimental treatment[a] (Days 1 to 48)
1	Commercial rat diet	SO + SP
2	Commercial rat diet	SO + CP
3	Commercial rat diet	BT + SP
4	Commercial rat diet	BT + CP

[a]SO + SP = soybean oil + isolated soy protein.
SO + CP = soybean oil + casein protein.
BT + SP = beef tallow + isolated soy protein.
BT + CP = beef tallow + casein protein.

Table 2. Sacrifice schedule (Number of animals sacrificed by dietary treatment and days on diet)

Dietary treatment[b]	Days on diet[a]											
	0	7	9	11	13	15	18	22	30	36	42	48
SO + SP	2	2	2	2	2	2	2	2	2	2	2	2
SO + CP	2	2	2	2	2	2	2	2	2	2	2	2
BT + SP	2	2	2	2	2	2	2	2	2	2	2	2
BT + CP	2	2	2	2	2	2	2	2	2	2	2	2

[a]Day rats were sacrificed.

[b]SO + SP = soybean oil + isolated soy protein.
SO + CP = soybean oil + casein protein.
BT + SP = beef tallow + isolated soy protein.
BT + CP = beef tallow + casein protein.

Experimental Animals and Housing

One hundred four adult male Sprague-Dawley rats[1] initially weighing between 300 and 325 g were used over a 48-day experimental period. Rats were caged individually in suspended wire mesh cages in a room kept at a temperature of 24 $\pm$ 3°C with a relative humidity of approximately 45%. A 12:12 reversed photoperiod was used for research convenience; the dark hours were from 6 AM to 6 PM and the light period was from 6 PM to 6 AM. Every two days, feed consumptions were recorded, feed containers were refilled, and cages were cleaned. A minimal light source was provided during cleaning of cages. Skin and respiratory conditions were monitored at the time of feeding. Animals were randomly assigned to

[1]Sasco Laboratories, Omaha, Nebraska 68108.

locations in the cage rack, to diets, and to days on treatment at sacrifice. Fresh water was provided daily. All rats were fed a stock laboratory diet from weaning to the beginning (day 1) of the experimental period.

Diets

Experimental diets

Four diets containing approximately equal amounts of either soybean oil (SO) or beef tallow (BT) and either isolated soy protein (SP) or casein protein (CP) were fed. The composition of the experimental diets is given in Table 3. Rats were fed the diets for one of 11 periods: 7, 9, 11, 13, 15, 18, 22, 30, 36, 42, or 48 days. There were two rats for each diet and period combination. Eight rats fed a stock laboratory diet were also included and these rats were sacrificed on day 0 to obtain baseline values for the cholesterol parameters measured. The eight remaining rats (which were fed the diets to provide replacements in case of animal loss) were not used.

Caloric densities of the diets are presented in Table 4. The diets were approximately isocaloric. Crystalline cholesterol[1] was dissolved in the fat (SO or BT) and comprised 0.2% of the experimental diets. All diets met the dietary requirements of the adult male rat. Feed intake was controlled so that each rat consumed approximately 10% of body weight per day.

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[1]Nutritional Biochemicals, Cleveland, Ohio 44128.

Table 3. Composition of experimental diets[a]

	Diets			
	Animal protein		Plant protein	
	Soy oil (%)	Beef tallow (%)	Soy oil (%)	Beef tallow (%)
Fat	19.05	19.05	19.05	19.05
Cholesterol	0.20	0.20	0.20	0.20
Casein	21.00	21.00	--	--
Isolated soy protein	--	--	21.75	21.75
Sucrose	29.39	29.39	28.59	28.59
Non-nutr-fiber (Solka-Floc)	3.02	3.02	3.02	3.02
Dextrin	19.99	19.99	19.99	19.99
DL-methionine	0.15	0.15	0.20	0.20
Vitamin mixture	2.00	2.00	2.00	2.00
Choline chloride	0.20	0.20	0.20	0.20
Mineral mixture	5.00	5.00	5.00	5.00
Total	100.00	100.00	100.00	100.00

[a]Supplied by Ralston Purina, Experimental Feeds Department, St. Louis, Missouri 63155.

Table 4. Caloric density of diets

Animal protein diet		Plant protein diet	
Protein:	21.00 g x 4 kcal = 84.00	21.75 g x 4 kcal =	87.00
Fat:	19.05 g x 9 kcal = 171.45	19.05 g x 9 kcal =	171.45
Carbohydrate:	49.38 g x 4 kcal = 197.52	48.58 g x 4 kcal =	194.32
Total kcal/100 g	= 452.97		452.77
Percent calories from fat	= 37.85		37.87
Percent calories from protein	= 18.54		19.22

Commercial rat diet

From the time of weaning until the initiation of the experimental diets, all rats were fed a stock laboratory diet[1] ad libitum; the diet's composition is listed in Table 5.

Table 5. Manufacturer's analysis of stock laboratory diet[a]

Item	Amount
Crude protein	24% minimum
Crude fat	4% minimum
Crude fiber	5% maximum

[a]Ingredients: ground yellow corn, soybean meal and bone scraps, wheat middlings, pulverized oats, ground whole wheat, dehydrated alfalfa, dried brewer's yeast, dry skim milk, cane molasses, animal fat (preserved with BHT, propylene glycol, citric acid), vitamin A palmitate, irradiated yeast (source of vitamin D_2), thiamine hydrochloride, riboflavin, pyridoxine hydrocholoride, calcium d-pantothenate, niacin, choline chloride, folic acid, vitamin B_{12}, d-alpha tocopheryl acetate, phosphate, 0.8% iodized salt, iron citrate, manganese oxide, copper oxide, cobalt carbonate, iron carbonate, zinc oxide, calcium iodate.

Tissue Analyses

Tissue collection

Rats were killed between 8 AM and 9 AM by cervical dislocation. Final body weights were determined just prior to killing the rats. Rats were laparotomized, pneumothoraxed, and terminal blood samples were obtained by cardiac puncture using a 22 gauge needle and syringe. The

[1]Teklad Mouse/Rat Diet, ARS/Sprague-Dawley Division of the Mogul Corporation, Winfield, Iowa 52659.

blood was placed in heparinized tubes, and plasma was separated by centrifugation.[1] Plasma was stored at 4°C until analyzed for cholesterol. All plasma samples were analyzed for cholesterol the same day they were collected.

Livers and small intestines were excised, blotted dry and weighed. The livers were placed in ice and the small intestines were placed in physiological saline for immediate preparation for incubation. Contents of the small intestine were removed before the intestine was weighed and placed in physiological saline.

Tissue preparation and incubation

The chilled liver from each animal was cut into ribbons approximately 2 mm thick. Liver slices 0.8 mm thick were then prepared on a tissue slicer.[2] The caudal five to six inches of the small intestine were separated from the rest of the small intestine and used to represent the ileum. The ileum was longitudinally sectioned to expose the brush border.

Approximately 300-mg aliquots of liver or small intestine were placed in 20 x 150 mm test tubes containing 5 ml of oxygenated Krebs bicarbonate buffer (pH 7.4) and a total concentration of 1 mM octanoic

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[1]Model RC2-B. Ivan Sorvall Incorporated, Norwalk, Connecticut 06856.

[2]Thomas Tissue Slicer. Arthur H. Thomas Co., Philadelphia, Pennsylvania 19104.

acid[1] containing 1 μCi of [1-^{14}]-octanoic acid.[2] Radiochemical purity of the [1-^{14}C]-octanoic acid was greater than 99% based on comparative chromatographic peak area measurements done by New England Nuclear, Boston, Massachusetts. Six tubes were incubated per tissue (liver and small intestine) from each animal. Three of the tubes were used for determinations of zero-time correction for radioactivity by killing the tissue immediately with 1.5 N H_2SO_4. The remaining three tubes were incubated for 90 minutes at 37°C in a metabolic shaker[3] at 160 oscillations/minute and then the tissue was killed with 1.5 N H_2SO_4. Dietschy and McGarry (1974) in previous experiments have shown that this slice thickness and shaker rate gave maximal rates of cholesterol synthesis, that the rates of incorporation of the [1-^{14}C]-octanoic acid into cholesterol are linear with respect to time during the 90-minute incubation, and that these concentrations of substrates give essentially V_{max} rates. Tissue and incubation media were stored at -17°C until analyzed for [1-^{14}C]-octanoic acid incorporation into digitonin precipitable sterols (DPS).

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[1]Octanoic acid. Sigma Chemical Co., St. Louis, Missouri 63178.

[2][1-^{14}C] octanoic acid, sodium salt. New England Nuclear, Boston, Massachusetts 02118.

[3]Dubnoff Metabolic Shaking Incubator. GCA/Precision Scientific, Chicago, Illinois 60647.

Determination of cholesterol biosynthesis

The method for determining the incorporation of [1-^{14}C]-octanoic acid into DPS has been described in detail by Siperstein and Guest (1966) and modified by Lowenstein et al. (1975). Briefly, this procedure may be outlined as follows: the contents of the incubation tubes were saponified by adding 5 ml of 5 N methanolic KOH and heating to 80°C for 1.5 hours, made up to a 50% ethanolic solution, and extracted three times with 10 ml of hexane to remove the nonsaponifiable lipids (primarily cholesterol). The hexane extracts were pooled and taken to dryness with nitrogen. The residue was redissolved in 5 ml of ethanol:acetone (1:1); then one drop of 10% acetic acid was added, followed by 3 ml of 0.5% digitonin[1] in 50% ethanol. After 24 hours, the β-hydroxy sterols were precipitated by centrifugation[2] as the digitonides, washed with 5 ml of acetone:ether (1:2), followed by a wash with 5 ml of diethyl ether, and then air dried. The digitonide pellet was then dissolved in 1.5 ml of hot 17 N glacial acetic acid and transferred to a counting vial. To this solution was added 15 ml of toluene containing PPO (0.3%) and POPOP (0.017%) for scintillation counting.

Each sample was counted in a Beckman LS 8000 series liquid scintillation system[3] equipped with an external standard. Cholesterogenesis in liver and small intestine was estimated by determining total DPMs of the

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[1]Digitonin. Sigma Chemical Company, St. Louis, Missouri 63178.

[2]International Centrifuge, Universal Model UV. International Equipment Company, Boston, Massachusetts 02118.

[3]Beckman Instruments, Inc., Scientific Instruments Division, Irvine, California 92713.

extracted tissue isolated as DPS. The cholesterol synthetic rates in the liver and small intestine are expressed as μmoles of [1-^{14}C]-octanoic acid incorporated into DPS per gram wet weight of tissue per hour incubation.

Plasma cholesterol

Cholesterol was determined within one day of drawing the blood using the enzymatic method of Allain et al. (1974). Enzyme kits containing cholesterol esterase and oxidase[1] were used to determine total cholesterol of plasma. The cholesterol esterase hydrolyzes the cholesterol esters to cholesterol and free fatty acids. The cholesterol is oxidized to 4-cholesten-3-one with the production of hydrogen peroxide. The latter reacts with 4-aminoantipyrine and phenol in the presence of peroxidases, forming a quiononeimine chromophore with intensity proportional to the total cholesterol in the sample.

The desiccated enzyme was reconstituted in 13.1 ml of distilled water and thoroughly mixed. Exactly 500 μl of enzyme were added to 10 μl of plasma, mixed, and heated for ten minutes at 37°C. The absorbance of the samples was read immediately by spectrophotometry[2] using a wavelength of 520 nm. The spectrophotometer was zeroed using a blank containing distilled water and enzyme. Standards were made using

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[1]CentrifiChem-Cholesterol Reagent/Enzymatic, Union Carbide Corporation, Rye, New York 10580.

[2]Gilford 2000 Spectrophotometer. Gilford Instrument Laboratories Inc., Oberlin, Ohio 44074.

recrystallized cholesterol dissolved in redistilled isopropanol and were refrigerated at 4°C. Moni-Trol[1] was used as a control. Total cholesterol concentration was determined by linear regression to compare samples to standards.

Tissue cholesterol

Tissue lipid extraction Approximately one gram each of liver and small intestine from each animal was weighed and extracted overnight on a wrist-action shaker[2] with 15 ml of chloroform:methanol (2:1). The supernatant was decanted through glass wool into a 50 ml test tube and the filtrate was made up to a volume of 15 ml with chloroform:methanol (2:1). Three ml of 0.73% NaCl were added to each tube, mixed, and samples were centrifuged[3] at 1500 rpm for 10 minutes. The upper layer was removed by suction and 3 ml of chloroform:methanol:water (3:48:47) containing 0.27% NaCl were added. The samples were mixed, centrifuged, and the former step repeated one more time. The washed extract was made to a final volume of 10 ml with chloroform:methanol:water (3:48:47) containing 0.27% NaCl.

Tissue cholesterol analysis Tissue cholesterol was determined by a modified enzymatic method of Carlson and Goldfarb (1977). Enzyme

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[1]Moni-Trol 1. Chemistry Control. DADE Division American Hospital Supply Corporation, Miami, Florida 33152.

[2]Model 75. Burrell Corporation, Pittsburgh, Pennsylvania 15219.

[3]International Centrifuge, Model CM. International Equipment Company, Boston, Massachusetts 02118.

kits containing cholesterol esterase and oxidase[1] were used to determine total cholesterol concentration of the liver and small intestine. The dried enzyme was reconstituted in 13.1 ml of distilled water and thoroughly mixed. Standards were prepared using recrystallized cholesterol dissolved in chloroform:methanol (2:1) and were refrigerated at 4°C. Moni-Trol was used as a control. Triplicate aliquots of lipid extract or standard (100 µl) were combined with Triton X-100[2] (50 µl) and air dried overnight. Triton X-100 was added to samples as a detergent to solubilize the extracted lipids.

The following day, exactly 500 µl of enzyme were added to samples and standards, mixed with a vortex, and incubated at 37°C for 30 minutes. Samples were read on a spectrophotometer[3] at 500 nm in microcuvettes. The spectrophotometer was zeroed using a blank containing distilled water and chloroform:methanol (2:1). Total cholesterol of liver and small intestine samples was compared to standards using linear regression and expressed as a percentage of the respective tissues.

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[1]CentrifiChem-Cholesterol Reagent/Enzymatic, Union Carbide Corporation, Rye, New York 10580.

[2]Triton X-100 No. T-6878. Sigma Chemical Company, St. Louis, Missouri 63178.

[3]Gilford 2000 Spectrophotometer. Gilford Instrument Laboratories Inc., Oberlin, Ohio 44074.

Statistical Analyses

The analysis of variance used to evaluate the data from 88 rats fed the experimental diets and sacrificed is shown in Table 6.

Table 6. Analysis of variance

Source of variation		d.f.
Treatments:		43
Protein	(1)	
Fat	(1)	
Protein x fat	(1)	
Day	(10)	
Protein x day	(10)	
Fat x day	(10)	
Protein x fat x day	(10)	
Rats within treatments		44
Total		87

The main effect of protein, fat and day and their interactions were determined for: serum and tissue cholesterol levels; liver and small intestine cholesterogenesis; feed consumption; and liver, small intestine and total body weight. Comparisons of the rats fed in these 44 treatment combinations with eight animals fed a stock laboratory diet were determined using the Student's t-test for unequal sample numbers.

RESULTS AND DISCUSSION

The parameters considered in the Results and Discussion fall into several major categories: 1) Tissue Cholesterol; 2) Tissue Cholesterol Biosynthetic Rate; 3) Body and Tissue Weights; and 4) Feed Consumption. Brief comments on the effects of feeding 0.2% cholesterol on tissue cholesterol and on adaptation and recovery times of plasma cholesterol concentration are discussed under the appropriate major category.

Tissue Cholesterol

Plasma

Plasma total cholesterol concentrations are shown in Table 7 and Figure 2. Values were derived from terminal blood samples taken by cardiac puncture.

Fat effect The mean total plasma cholesterol concentrations of rats fed diets containing saturated fat (BT) were higher than those of rats fed diets containing polyunsaturated fat (SO), 85 versus 77 mg/dl ($P < .05$) (Table 7). The BT- and SO-fed rats had significantly lower ($P < .05$) plasma cholesterol when the diet included SP rather than CP as the source of dietary protein (Table 7). The response was greater, however, in the BT-fed rats. There was no significant interaction between dietary fat and protein source on total plasma cholesterol concentration.

The highest total plasma cholesterol levels, of the experimental treatments, were in rats fed saturated fat (BT) plus animal protein

Table 7. Plasma total cholesterol concentrations (mg/dl)

Dietary treatment	Days on experimental diet[a]												
	0[b]	7	9	11	13	15	18	22	30	36	42	48	Means[c]
SO + SP		91[d]	69	67	69	77	85	89	91	71	51	52	74
SO + CP		65	84	52	102	75	90	123	94	68	72	62	81
BT + SP		71	78	76	79	82	101	95	89	57	68	67	78
BT + CP		94	114	77	86	91	105	92	110	73	98	69	92
Means[e]	66	80	86	68	84	81	95	100	96	67	72	62	

Standard error of the diet mean is ± 3.16.
Standard error of the day mean is ± 5.24.

[a]Days on experiment at time of sacrifice.

[b]The eight rats sacrificed at day 0 were fed a commercial rat diet.

[c]Diet mean plasma cholesterol concentrations for rats sacrificed at 7 to 48 days.

[d]Mean plasma cholesterol concentrations of two animals (two rats were sacrificed from each dietary group at each of days indicated).

[e]Day mean plasma cholesterol concentrations.

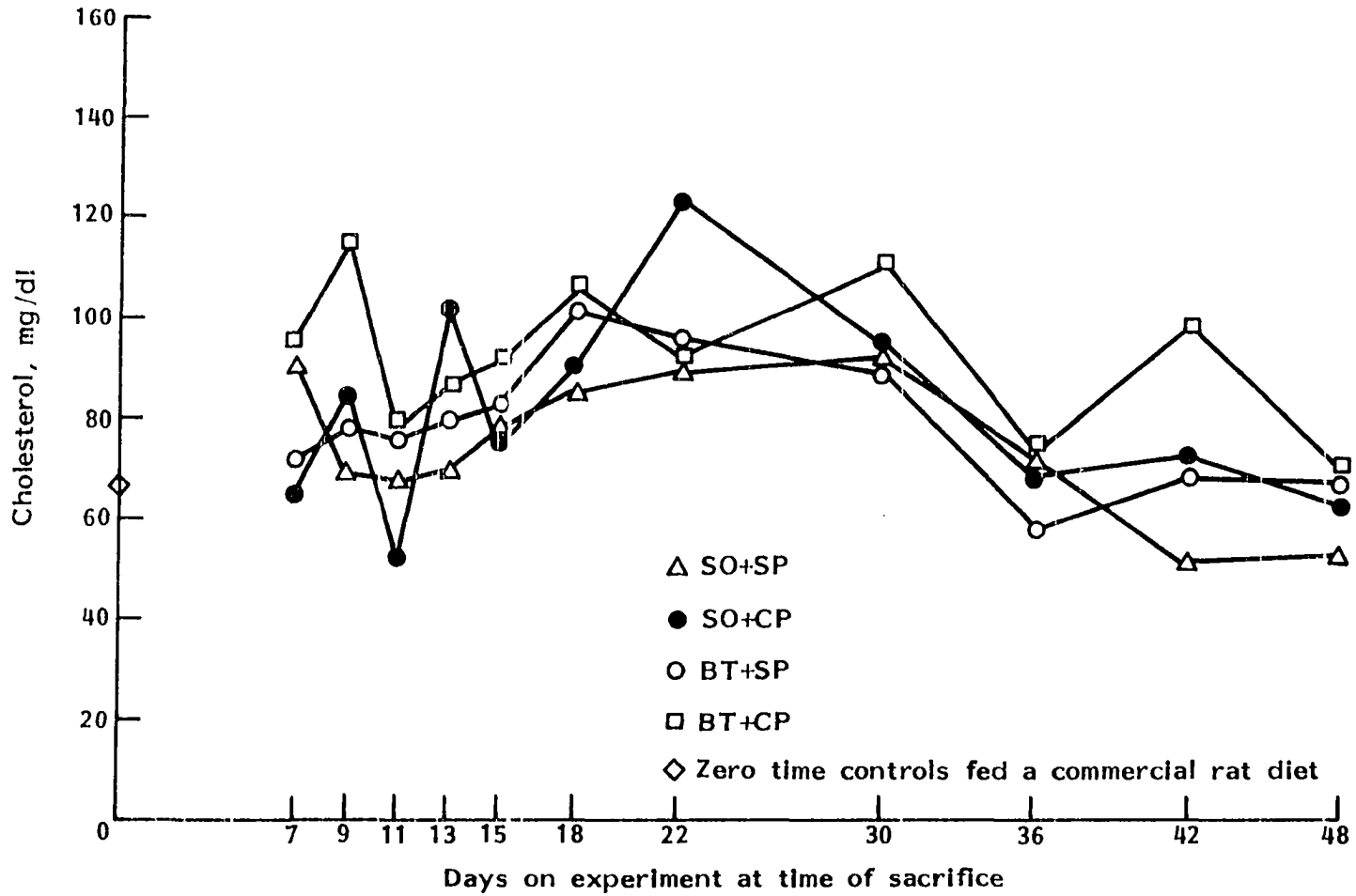

Figure 2. Plasma total cholesterol concentrations

(CP) (92 mg/dl) and the lowest values were in the unsaturated fat (SO) plus plant protein (SP) group (74 mg/dl). These results support those of Forsythe et al. (1980) who found that pigs fed animal protein plus saturated fat had higher total plasma cholesterol (205 mg/dl) than pigs fed plant protein plus polyunsaturated fat (111 mg/dl).

Data from the present study indicate that plasma cholesterol concentration is influenced by degree of saturation of dietary fat, because plasma cholesterol levels were lower in rats fed polyunsaturated fat. Many past studies have shown that polyunsaturated fats have a hypocholesterolemic effect. Results similar to those of the present study have been found in studies with pigs (Forsythe et al., 1979; Julius and Wiggers, 1979), rabbits (Kritchevsky and Tepper, 1968; Minick and Murphy, 1973), and man (Stamler, 1978; Tan et al., 1980).

Forsythe et al. (1979) found that plasma total cholesterol was elevated 30% throughout a 14-week experimental period in pigs fed saturated fat (tallow) as compared with pigs fed polyunsaturated fat (corn oil). Greer et al. (1966) reported that plasma cholesterol levels decreased by 25 mg/dl in pigs fed SO versus BT. One reason for the greater treatment response in the experiment of Forsythe et al. (1979) than observed in the present experiment may have been the sources of fats and species used.

Kritchevsky and Tepper (1968) and Minick and Murphy (1973) produced hypercholesterolemia and atherosclerotic lesions in rabbits by feeding cholesterol-free, semisynthetic diets containing saturated fat. Similar responses have been reported with humans; i.e., diets rich in saturated

fats tended to elevate plasma cholesterol, while diets containing unsaturated fat tended to lower it (Stamler, 1978). Tan et al. (1980) found that saturated fat diets increased serum cholesterol 23% in six normolipidemic subjects.

The mechanism by which polyunsaturated fats reduce plasma cholesterol concentrations has not been elucidated. Possible mechanisms are: 1) an increase in fecal excretion of neutral and acid sterols, thus producing a secondary decrease of plasma cholesterol (Connor et al., 1969; Moore et al., 1968; Nestel et al., 1975); 2) a shift in cholesterol from the plasma to tissue pools (Biederdorf and Wilson, 1965; Bloomfield, 1964; Grundy, 1975); 3) a decrease in cholesterol absorption and endogenous cholesterol synthesis; and 4) an alteration of lipoprotein composition and metabolism.

Protein effect The mean total plasma cholesterol concentrations were higher in rats fed diets containing animal protein (CP) than in those fed diets containing plant protein (SP), 86 versus 76 mg/dl ($P < .005$) (Table 7). In this study, protein had a greater effect on plasma total cholesterol concentrations than fat.

The source of dietary fat had an effect on plasma cholesterol levels in both CP- and SP-fed rats. The CP- and SP-fed rats had significantly lower ($P < .05$) plasma total cholesterol concentrations with SO than with BT as the source of dietary fat (Table 7). Sirtori et al. (1979) suggested that the hypocholesterolemic effect of soy protein is reduced when fed with saturated fat; the present study supports this conclusion. Forsythe et al. (1980), however, found that feeding diets with highly

saturated fats to pigs did not reduce the effectiveness of plant protein in lowering plasma cholesterol concentrations.

Other studies support our results that protein derived from animal sources produces a hypercholesterolemic response in contrast to proteins derived from plant sources. The SP diet has been described as hypocholesterolemic compared to CP and other animal proteins in rabbits (Carroll, 1978) and rats (Yadav and Liener, 1977) fed low-fat diets without added cholesterol. Studies with humans further confirm the hypocholesterolemia associated with dietary proteins of plant origin compared to those of animal origin (Carroll et al., 1978b; Sirtori et al., 1977). Carroll et al. (1978b) reported that plasma cholesterol levels of humans decreased by 16 mg/dl when SP replaced a mixture of animal proteins (meat and casein) in high fat diets. Sirtori et al. (1979) also reported that hypercholesterolemic patients fed a mixture of plant proteins (63% SP) in diets containing 25% energy as fat had plasma cholesterol levels that averaged 50 mg/dl lower than those of patients fed a mixture of plant and animal proteins. Forsythe et al. (1980) and Julius and Wiggers (1979) found that pigs fed animal protein diets had higher plasma cholesterol levels than pigs fed plant proteins.

Neves et al. (1980) suggested that the hypocholesterolemic effects of plant proteins reported in the literature may be limited to specific experimental conditions. Plant proteins seemed to have the most hypocholesterolemic effect in rats already made hypercholesterolemic with cholesterol-rich and saturated fatty acid-rich diets (Carroll et al., 1979; Yadav and Liener, 1977). The moderate level of fat (19%) and

cholesterol (0.2%) used in the present study, therefore, may account for the lower response to treatment as compared to other investigations reported in the literature. The hypocholesterolemic effect of plant proteins seemed to be directly related to the plasma cholesterol level of the animal (Roy and Schneeman, 1981; Sirtori et al., 1977).

Several investigations have proposed mechanisms by which dietary plant protein lowers plasma total cholesterol concentrations. Carroll (1978) found that the different amino acid compositions between plant and animal protein are responsible, to some extent, for the hypocholesterolemia observed with plant protein sources. Kritchevsky et al. (1978) demonstrated that SP was less atherogenic than casein for rabbits fed a semipurified, cholesterol-free diet. The authors attributed the difference between these proteins to their ratios of arginine/lysine (0.49 in CP; 1.18 in SP). Conversely, Hevia et al. (1980) found that the hypercholesterolemia found with CP feeding was unrelated to the high lysine content of this protein. Gibney et al. (1979) and Sautier et al. (1979) demonstrated that the hypocholesterolemic response to SP cannot be attributed to its saponin content. Roy and Schneeman (1981) demonstrated increased bile acid secretion in mice fed SP, leading to increased cholesterol catabolism and lowered plasma cholesterol levels.

Commercial rat diet The mean plasma cholesterol concentration of rats fed the SO + SP diet over the 48-day period was not significantly different from that of rats sacrificed at 0 time. Rats fed diets SO + CP and BT + SP, however, were significantly different ($P < .05$) from rats fed the commercial diet having 15 and 12 mg/dl, respectively, higher

mean plasma cholesterol concentrations. Furthermore, rats fed the BT + CP diet had the highest plasma cholesterol concentration which averaged 26 mg/dl greater than the plasma cholesterol concentration of rats fed the commercial diet.

The addition of 0.2% cholesterol to the experimental diets may be responsible for the higher plasma cholesterol levels in rats fed these diets versus rats fed the commercial diet. Studies with experimental animals have consistently demonstrated an increased plasma cholesterol concentration with increased dietary cholesterol (Glueck and Connor, 1978). Connor et al. (1961b) and Mattson et al. (1972) with humans and Kenealy et al. (1977) with goats demonstrated that the level of ingestion of cholesterol had a significant effect on the plasma cholesterol levels.

A rise in plasma total cholesterol concentrations during the first half of the experimental period was found in all dietary groups (Figure 2). By day 48, however, plasma total cholesterol concentrations had decreased to approximately the initial levels (of the rats fed only the commercial diet). The reason for the initial elevation and subsequent drop of plasma cholesterol levels is not clear. The addition of 0.2% crystalline cholesterol to the experimental diets may be responsible for the initial rise. The mechanism whereby rats recovered from the addition of dietary cholesterol, by demonstrating a drop in plasma cholesterol concentration, is not known. The rats appeared, however, to adapt to cholesterol feeding through a sensitive control mechanism aimed at maintaining blood cholesterol regardless of dietary manipulations. Forsythe

et al. (1980) observed an initial elevation of plasma cholesterol levels which peaked at four weeks in pigs fed experimental diets supplemented with 0.6 mg/kcal of crystalline cholesterol. An elevation in plasma cholesterol concentrations was not observed in control pigs unsupplemented with cholesterol. At six weeks, plasma cholesterol levels had dropped to control levels, suggesting that pigs also adapt to the addition of dietary cholesterol. The authors observed, however, that after eight weeks on the experimental diets, plasma total cholesterol levels rose and repeaked at 12 weeks.

Liver

Liver cholesterol levels are shown in Table 8 and Figure 3.

Fat effect The SO-fed rats had a mean liver cholesterol (%) significantly higher ($P < .0001$) than BT-fed rats. Means for liver cholesterol over the 48-day experimental period were 0.52 and 0.34% with these treatments, respectively. The source of dietary protein had no significant effect on liver cholesterol in both BT- and SO-fed rats (Table 8).

Other authors have found evidence that polyunsaturated fats fed to animals with supplemental cholesterol increased hepatic cholesterol concentrations compared to saturated fats in guinea pigs (Crocker et al., 1979), rabbits (Hough and Bassett, 1975) and rats (Kellogg, 1974; McGovern and Quakenbush, 1973a; Rankins, 1973; Wiggers et al., 1977b). Egwin and Kummerow (1972) fed male weanling rats for 15 weeks diets containing 20% fat from corn oil or BT. Liver cholesterol levels of corn oil-fed animals exceeded those of rats fed BT by 50%. In the present study, male rats were fed a 19% fat diet for approximately 7 weeks and

Table 8. Liver cholesterol (%)

Dietary treatment	Days on experimental diet[a]											Means[c]
	0[b]	7	9	11	13	15	18	22	30	36	42	48	
SO + SP		0.33[d]	0.27	0.43	0.72	0.42	0.42	0.43	0.50	0.91	0.75	0.41	0.51
SO + CP		0.34	0.34	0.41	0.68	0.41	0.44	0.42	0.97	0.60	0.68	0.62	0.54
BT + SP		0.26	0.25	0.29	0.41	0.35	0.29	0.29	0.28	0.38	0.27	0.39	0.31
BT + CP		0.35	0.44	0.24	0.56	0.45	0.28	0.37	0.54	0.31	0.30	0.37	0.38
Means[e]	0.21	0.32	0.32	0.34	0.59	0.40	0.36	0.38	0.57	0.55	0.50	0.45	

Standard error of the diet mean is ± 0.38.
Standard error of the day mean is ± 0.63.

[a]Days on experiment at time of sacrifice.

[b]The eight rats sacrificed at day 0 were fed a commercial rat diet.

[c]Diet mean liver cholesterol for rats sacrificed at 7 to 48 days.

[d]Mean liver cholesterol of two animals (two rats were sacrificed from each dietary group at each of days indicated).

[e]Day mean liver cholesterol.

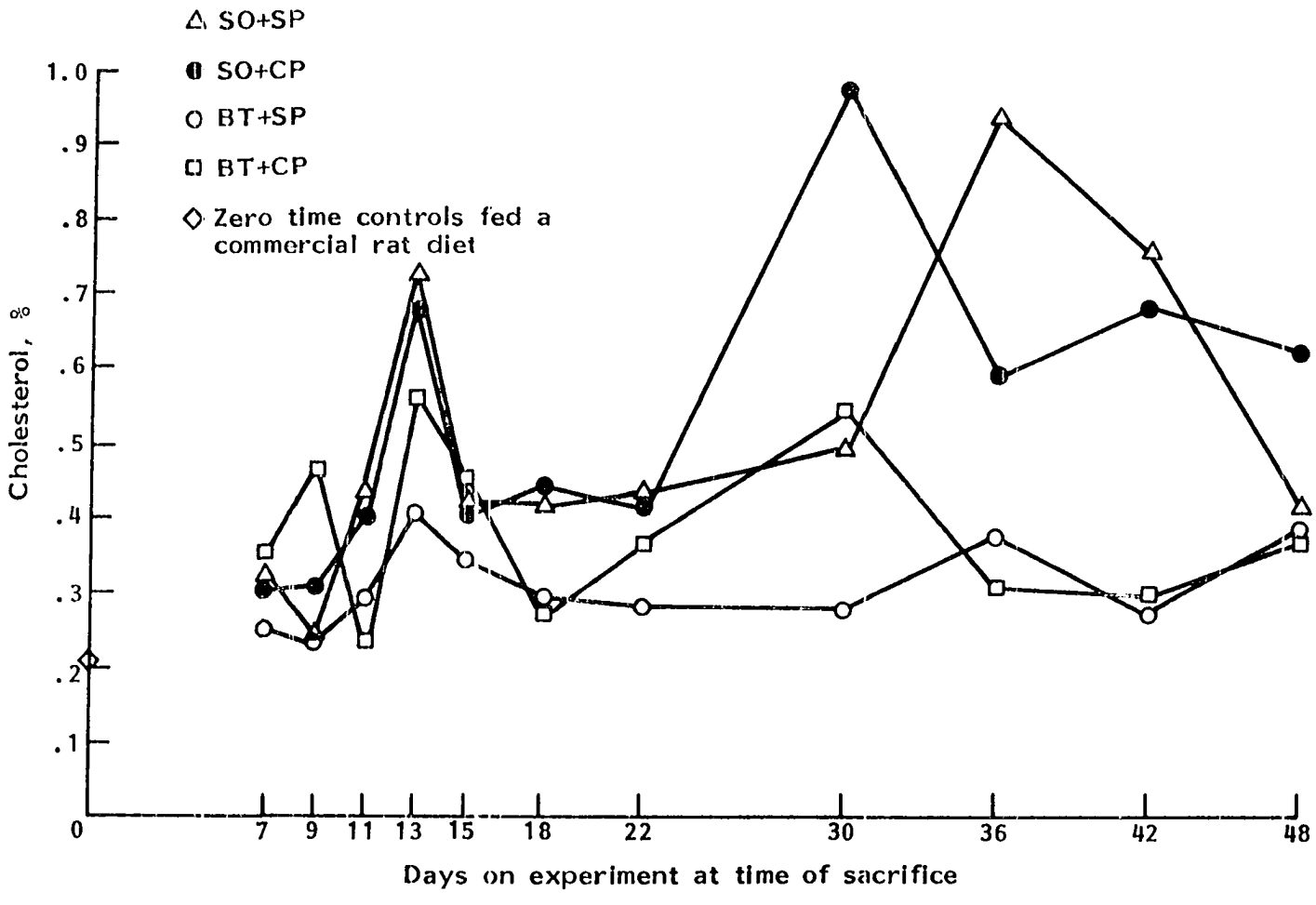

Figure 3. Liver cholesterol

the SO-fed animals had 52% greater liver cholesterol than BT-fed rats.

Ho et al. (1974) demonstrated that the propensity toward dietary cholesterol accumulation in tissues is determined by degree of hypercholesterolemia and duration of exposure. In the present study, the amount of dietary cholesterol was not great enough to make the animals severely hypercholesterolemic; therefore, dietary cholesterol probably did not accumulate in the liver of these rats. Ide et al. (1980) found that the deposition of cholesterol in the liver microsomal fraction was noted only when rats were fed high levels of cholesterol. O'Brien and Reiser (1979) demonstrated no effect of dietary cholesterol on liver cholesterol of rats which ate cholesterol-containing, human-type diets.

Data from the present study indicate that transport of cholesterol within the serum-hepatic pool is influenced by degree of saturation of dietary fat, i.e., polyunsaturated fat decreased plasma cholesterol (Table 7) and increased liver cholesterol (Table 8). A shift of serum-hepatic cholesterol equilibrium toward liver has been previously attributed to polyunsaturated fat feeding to rats, with and without dietary cholesterol supplementation (Biederdorf and Wilson, 1965; Bloomfield, 1964; Grundy, 1975). The present study supports the theory that polyunsaturated fats effect a redistribution of cholesterol within the body with a shift of cholesterol from plasma to tissue pools.

Results of this study provide evidence that differences in liver cholesterol between SO- and BT-fed rats increased significantly ($P < .01$) with time (Figure 3). The differences between liver cholesterol of SO- and BT-fed rats were more marked at the end compared to the

beginning of the 48-day experimental period, producing a fat x day interaction. One possible explanation for the interaction is that the SO group stored a greater proportion of cholesterol in the liver with time compared to the BT-fed animals.

Protein effect The mean liver cholesterol was higher in rats fed diets containing CP than in rats fed diets containing SP, 0.46 versus 0.41% ($P < .05$) (Table 8). Results demonstrate that protein source had less effect on liver cholesterol than fat source. The source of dietary fat had an effect on liver cholesterol in both CP- and SP-fed rats. The CP- and SP-fed rats had significantly higher ($P < .05$) liver cholesterol levels with SO than with BT as the source of dietary fat (Table 8).

In a similar study, Forsythe et al. (1980) examined the effects of dietary protein and fat sources on cholesterol parameters in pigs. Neither of the experimental treatments, fat or protein, caused any change in liver cholesterol content. Kritchevsky et al. (1978) also found that liver cholesterol concentrations were comparable when rabbits were fed a semipurified, cholesterol-free diet containing SP or CP. One reason for the greater treatment response in the present experiment than that observed in the experiment of Forsythe et al. (1980) may have been the sources of plant and animal proteins used. SP and CP were the sole sources of plant and animal protein, respectively, in our study whereas Forsythe et al. (1980) fed a mixture of plant proteins (soy, 50%; wheat, 25%, corn, 25%) and milk proteins (casein, 90%, lactalbumin, 10%). At least in rabbits, wheat protein was found to increase liver cholesterol compared to SP (Carroll and Hamilton, 1975). The plant protein diet of

Forsythe et al. (1980), therefore, would elevate liver cholesterol resulting in a smaller difference in liver cholesterol levels between plant and animal protein fed rats. The present experiment, however, adds support to the results of Julius (1980) who found that liver cholesterol concentrations of CP-fed pigs were significantly greater ($P < .01$) than liver cholesterol of SP-fed pigs.

Commercial rat diet The mean liver cholesterol of rats fed experimental diets was not statistically significantly different from the means of the rats killed at 0 time. The rats sacrificed from days 30 to 48, however, did tend to have a greater percentage of cholesterol in the liver compared to rats killed at 0 time. Increased liver cholesterol accumulation of polyunsaturated fat-fed rats (over that of saturated fat-fed rats) has been shown to be due to an increased accumulation of cholesterol esters (Bloomfield, 1964; Kellogg, 1974). Bloomfield (1964) has shown safflower-fed rats to have a longer blood cholesterol half-life and to have a larger blood-liver cholesterol pool than butter-fed rats.

Also, different species of animals seem to vary considerably in their ability to accumulate cholesterol. In the rat, cholesterol feeding causes only small increases in tissue cholesterol concentration (Ho and Taylor, 1968). Dietschy and Siperstein (1967) found that any tendency for the rat to accumulate absorbed dietary cholesterol is compensated by a rapid inhibition of cholesterol synthesis and by enhanced excretion of bile acids. These two homeostatic mechanisms are so efficient that the total body exchangeable pool remains constant at a low level in the rat.

Small intestine

Cholesterol levels in the wall of the small intestine are shown in Table 9 and Figure 4.

Fat effect The small intestinal cholesterol level was essentially the same for rats on all diets. Crocker et al. (1979) demonstrated that intestinal cholesterol concentrations of guinea pigs were similar regardless of dietary fat composition. Carlson (1975) showed a tendency for safflower oil to increase small intestinal cholesterol concentrations in rats, but the difference between safflower oil and BT were statistically not significant. In an earlier rat study, however, supplementation of a low-fat diet with two or ten percent corn oil for one year increased cholesterol concentration in the gastrointestinal tract (Gerson et al., 1961).

Protein effect Intestinal (ileal) cholesterol levels showed no significant difference between CP- and SP-fed rats (Table 9). No study could be found in the literature that dealt with the effect of dietary protein source on small intestinal cholesterol content.

Commercial rat diet The mean small intestinal cholesterol of rats on the experimental diets was not significantly different from the means of eight rats (on the commercial diet) sacrificed at 0 days (Figure 4). The rats sacrificed from days 30 to 48, however, did tend to have a greater percentage of cholesterol in the intestinal wall compared to rats sacrificed at 0 time. Therefore, the addition of 0.2% cholesterol to the experimental diets, on the average, was not enough to produce an accumulation of excess cholesterol in the small intestine. Peng et al.

Table 9. Small intestine cholesterol (%)

Dietary treatment	Days on experimental diet[a]												
	0[b]	7	9	11	13	15	18	22	30	36	42	48	Means[c]
SO + SP		0.18[d]	0.21	0.26	0.28	0.27	0.39	0.26	0.44	0.33	0.25	0.32	0.29
SO + CP		0.23	0.28	0.23	0.32	0.23	0.38	0.26	0.42	0.33	0.28	0.31	0.30
BT + SP		0.24	0.28	0.24	0.28	0.30	0.35	0.24	0.35	0.32	0.23	0.34	0.29
BT + CP		0.27	0.18	0.28	0.30	0.31	0.38	0.31	0.45	0.34	0.26	0.43	0.32
Means[e]	0.22	0.23	0.24	0.25	0.29	0.28	0.37	0.27	0.41	0.33	0.25	0.35	

Standard error of the diet mean is $\pm$ 0.12.
Standard error of the day mean is $\pm$ 0.19.

[a]Days on experiment at time of sacrifice.

[b]The eight rats sacrificed at day 0 were fed a commercial rat diet.

[c]Diet mean small intestine cholesterol for rats sacrificed at 7 to 48 days.

[d]Mean small intestine cholesterol of two animals (two rats were sacrificed from each dietary group at each of days indicated).

[e]Day mean small intestine cholesterol.

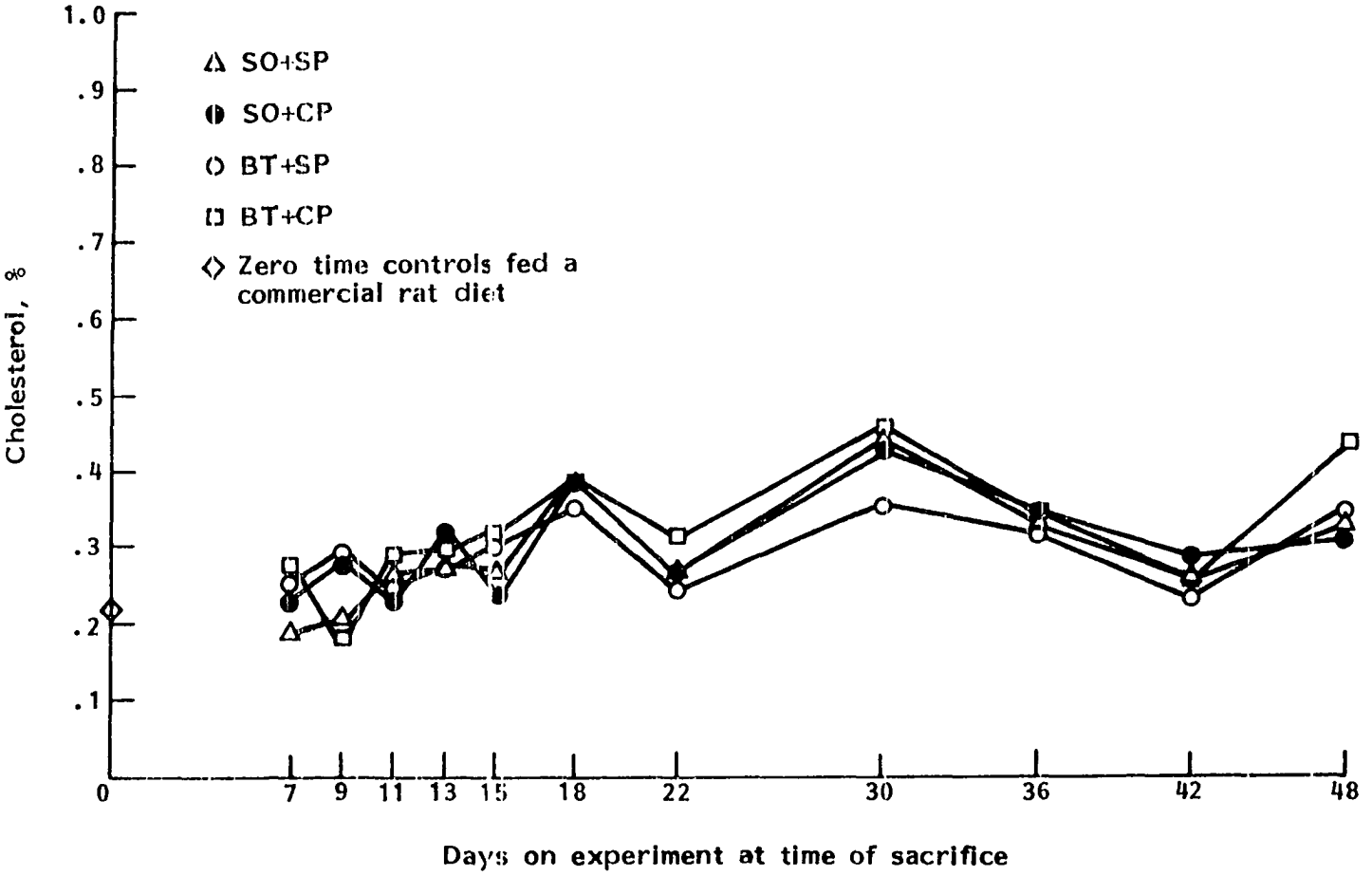

Figure 4. Small intestine cholesterol

(1974) found no excessive accumulation of cholesterol in the intestinal mucosa of rats after five months of feeding a 2% cholesterol supplemented diet.

Tissue Cholesterol Synthesis

Liver

The rates of liver cholesterol biosynthesis (μmoles/hour x g tissue) are shown in Table 10 and Figure 5.

Fat effect The rate of liver cholesterol synthesis was essentially the same for all experimental diets. Reports on the influence of saturated versus unsaturated fat on the synthesis of cholesterol have been conflicting. The observation that unsaturated dietary fat (SO) does not elevate hepatic cholesterogenesis (Table 10) is surprising in view of the fact that studies have indicated elevated cholesterol biosynthesis with substitution of polyunsaturated fat for saturated fat sources. Cholesterol synthesis in liver slices (Hill et al., 1960), in perfused liver (Goh and Heimberg, 1973) or *in vivo* (Dupont, 1966a) all demonstrated an increased hepatic cholesterogenesis following treatment with unsaturated fat in ad libitum fed rats. Dupont (1966a) demonstrated that corn oil, when compared with BT, increased cholesterol synthesis approximately tenfold. Carroll (1964) found dietary fats stimulated cholesterogenesis and unsaturated fats were more effective than saturated fats.

Other reports, however, indicated that there is no significant change in hepatic cholesterol synthesis due to saturation of dietary fat

Table 10. Liver cholesterol biosynthesis (μmoles/hour x g tissue)

Dietary treatment	Days on experimental diet[a]												
	0[b]	7	9	11	13	15	18	22	30	36	42	48	Means[c]
SO + SP		2.60[d]	0.84	1.31	0.54	0.87	0.56	0.94	0.79	0.40	0.65	0.35	0.89
SO + CP		1.41	1.18	0.99	0.55	0.61	0.21	0.28	0.40	1.04	0.49	0.32	0.66
BT + SP		1.20	1.46	0.25	0.62	1.27	2.18	0.64	0.56	0.43	0.79	0.41	0.89
BT + CP		0.86	1.82	1.00	1.32	0.58	0.58	0.08	1.05	0.79	0.92	0.23	0.86
Means[e]	1.70	1.52	1.32	0.89	0.76	0.83	0.88	0.48	0.70	0.66	0.71	0.33	

Standard error of the diet mean is ± 0.15.
Standard error of the day mean is ± 0.24.

[a]Days on experiment at time of sacrifice.

[b]The eight rats sacrificed at day 0 were fed a commercial rat diet.

[c]Diet mean liver cholesterol biosynthetic rates for rats sacrificed at 7 to 48 days.

[d]Mean liver cholesterol biosynthetic rates of two animals (two rats were sacrificed from each dietary group at each of days indicated).

[e]Day mean liver cholesterol biosynthetic rates.

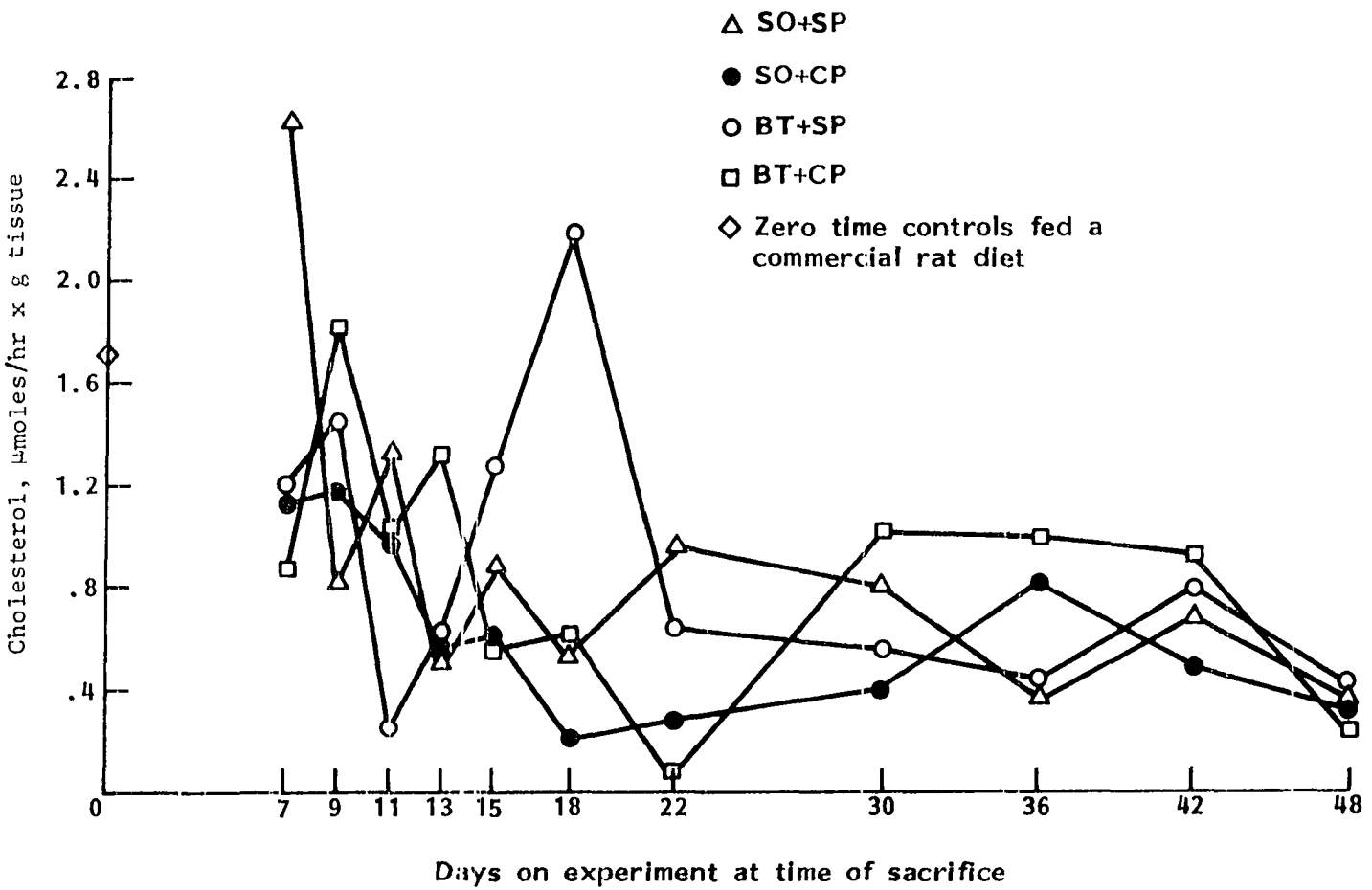

Figure 5. Liver cholesterol biosynthetic rates

source. Carlson et al. (1978) found that safflower oil, compared with BT, did not elevate hepatic cholesterogenesis in adult rats. Hepatic cholesterol synthesis in safflower oil-fed and BT-fed rats was similar. Furthermore, another study (male rats) with dietary safflower oil and BT, employing several labeled precursors of cholesterol, also failed to demonstrate an increase in cholesterol biosynthesis with the more unsaturated fat (Spritz et al., 1965). Bochenek and Rodgers (1978) studied endogenous cholesterol synthesis in the rat liver after animals had been on various dietary programs for a period of four weeks. These authors also found that the source of dietary fats, saturated versus unsaturated, produced no change in hepatic cholesterol synthesis.

The level of dietary fat may determine differences in rates of hepatic cholesterogenesis when saturation of dietary fat is a variable. One of the nutritional factors controlling the activity of HMG-CoA reductase in the liver is the amount of dietary fat. One might speculate that with consumption of diets containing 19% fat, as used in the present study, NADPH would cease to be limiting for maximal activation of HMG-CoA reductase. Reports that increased concentrations of NADPH can maximally activate HMG-CoA reductase (Tormanen et al., 1975) would support this speculation.

Protein effect No significant difference in the rate of liver cholesterol biosynthesis was observed between CP- and SP-fed rats (Table 10).

One study was found in the literature which documented the effect of protein source on hepatic cholesterol biosynthesis. Reiser et al. (1977)

found that the substitution of SP for CP in a semipurified diet elicited a small but significant increase in HMG-CoA reductase activity. The observed increase in HMG-CoA reductase activity corresponded to an increase in hepatic cholesterogenesis on the SP diet. In the present study, hepatic cholesterol synthesis tended to be elevated in the SP-fed rats (0.89 μmoles/hour x g tissue) compared to the CP-fed rats (0.76 μmoles/hour x g tissue) but the difference was not statistically significant. Dietary protein level has been shown not to affect hepatic cholesterol synthesis in the rat. Carlson (1975) demonstrated that dietary protein concentration did not influence synthesis of hepatic cholesterol. Evidence provided by the present study showed that the hypocholesterolemic effect of plant protein is not related to decreased hepatic cholesterogenesis.

Commercial rat diet The mean hepatic cholesterol synthetic rates of all rats fed the experimental diets were significantly less ($P < .05$) than the rates of 0 time rats fed the commercial diet. Synthesis rates by rats fed diet SO + CP, however, were the most different ($P < .005$) from those of rats sacrificed on day 0 with the lowest hepatic cholesterol synthetic rate of any diet (Figure 5). Generally, the liver cholesterol synthetic rate of rats fed the experimental diets decreased over the 48-day experimental period.

The most plausible explanation for the lower hepatic cholesterogenesis in rats fed experimental diets is that the 0.2% dietary cholesterol decreased the activity of HMG-CoA reductase, the primary rate-limiting enzyme in cholesterol biosynthesis. In rat liver, 0.2%

cholesterol feeding resulted in an average reduction in cholesterol synthetic rate from 1.70 to 0.82 μmoles/hour x g tissue. In these rats, the dietary cholesterol led to a negative feedback inhibition on hepatic cholesterol synthesis of approximately 50%. The rats fed the experimental diets, however, were 48 days older than the commercial diet fed rats which could explain some of the difference in hepatic cholesterogenesis. Ho and Taylor (1970) found that feeding cholesterol produced a feedback inhibition of 80 to 96% in rats, while in monkeys and man, suppressions were less apparent at 60-80% and 50%, respectively. The rats in Ho and Taylor's (1970) study, however, were fed higher levels of cholesterol than rats in the present study. Hepatic cholesterol synthesis was found to be under negative feedback control by the amount of dietary cholesterol intake in rats (Siperstein and Guest, 1966), squirrel monkeys (Dietschy and Wilson, 1968), and man (Siperstein, 1970).

The rate of cholesterogenesis was also found to decrease in response to increases in dietary cholesterol due to the synthesis of reductase inhibitors. Rodwell et al. (1976) suggested the inhibitors were transitory, acting only until a decrease in synthesis was initiated. Reductase activity may also be mediated through cAMP and Mg-ATP-dependent inactivation systems (Raskin et al., 1974).

Small intestine

The rates of small intestinal (terminal ileum) cholesterol biosynthesis (μmoles/hour x g tissue) are shown in Table 11 and Figure 6. Cholesterol synthesis occurs throughout the length of the small intestine. Several studies, however, have demonstrated that the terminal ileum is

Table 11. Small intestine cholesterol biosynthesis (μmoles/hour x g tissue)

Dietary treatment	Days on experimental diet[a]												
	0[b]	7	9	11	13	15	18	22	30	36	42	48	Means[c]
SO + SP		0.01[d]	1.05	0.54	1.02	0.85	0.57	0.12	0.32	0.33	0.14	0.33	0.48
SO + CP		0.01	0.49	0.38	0.62	0.45	0.31	0.47	0.37	0.81	0.37	0.07	0.40
BT + SP		0.38	0.33	0.32	0.79	0.54	0.51	0.57	0.67	0.55	0.30	0.57	0.50
BT + CP		0.31	2.16	0.58	1.88	1.01	1.61	0.65	0.73	0.83	1.13	0.15	1.00
Means[e]	1.72	0.18	1.00	0.45	1.08	0.71	0.75	0.45	0.52	0.63	0.48	0.28	

Standard error of the diet mean is ± 0.07.
Standard error of the day mean is ± 0.13.

[a]Days on experiment at time of sacrifice.

[b]The eight rats sacrificed at day 0 were fed a commercial rat diet.

[c]Diet mean small intestine cholesterol biosynthetic rates for rats sacrificed 7 to 48 days.

[d]Mean small intestine cholesterol biosynthetic rates of two animals (two rats were sacrificed from each dietary group at each of days indicated).

[e]Day mean small intestine cholesterol biosynthetic rates.

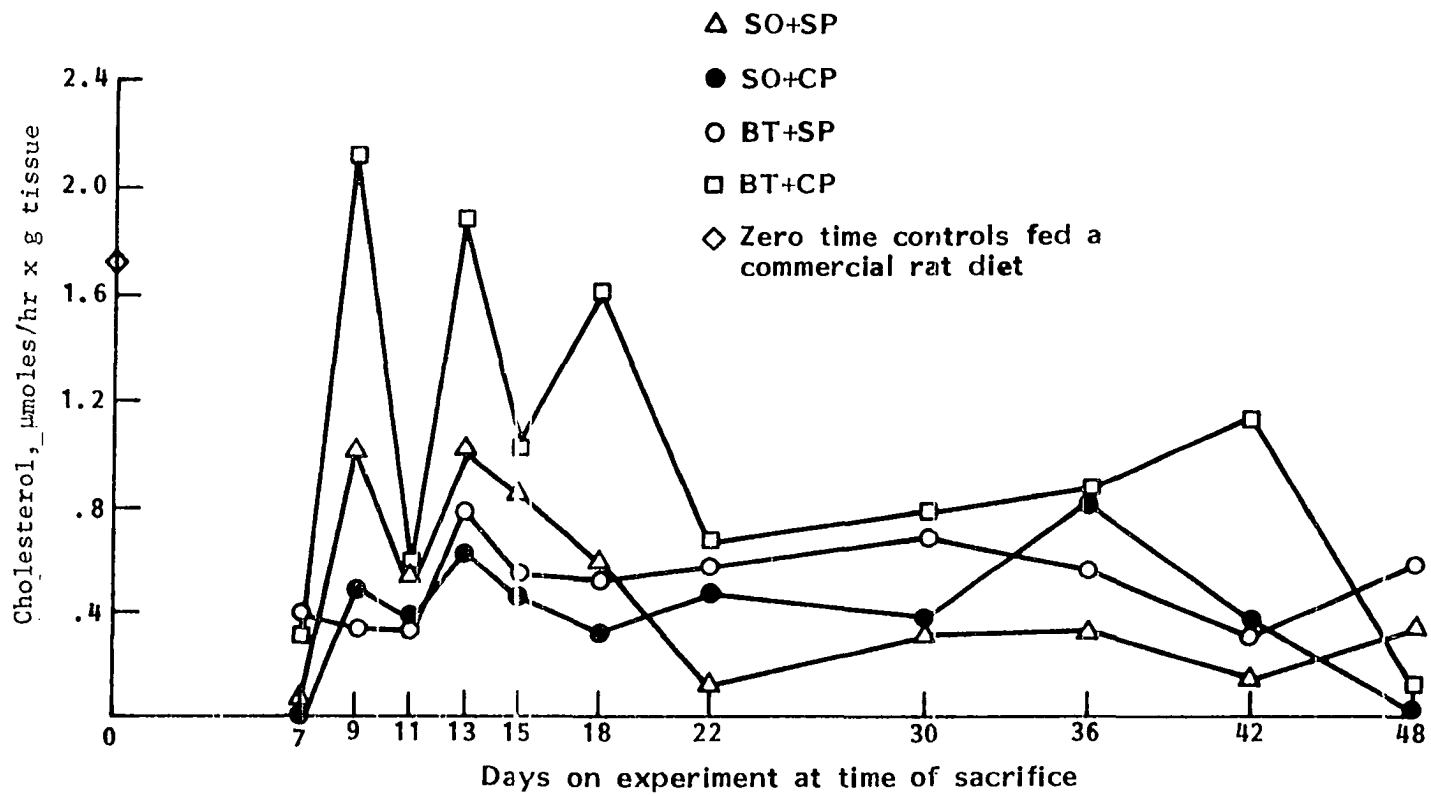

Figure 6. Small intestine cholesterol biosynthetic rate

the major site of intestinal cholesterogenesis (Dietschy and Gamal, 1971; Dietschy and Siperstein, 1965; Shefer et al., 1972a, b). In the present study, small intestine (Table 11) and liver (Table 10) converted approximately the same amounts of octanoic acid to DPS. Liepa (1976) demonstrated approximately equal relative rates of acetate or glucose incorporation into cholesterol by liver and small intestine in the goat.

Fat effect The mean intestinal cholesterol synthetic rates were higher in rats fed diets containing saturated fat (BT) than rats fed diets containing polyunsaturated fat (SO), 0.75 versus 0.44 μmoles/hour x g tissue ($P < .001$). The source of dietary protein had no effect on small intestinal cholesterogenesis in SO-fed rats. However, the BT-fed rats had significantly higher ($P < .001$) small intestinal cholesterol synthetic rates with CP than with SP as the source of dietary protein. Examination of Table 11 reveals that the mean small intestinal cholesterol synthetic rates are approximately equal in rats on all experimental diets except for the BT + CP diet. The BT + CP diet produced twice the synthetic rate compared to the other experimental diets, resulting in a significant ($P < .01$) fat x protein interaction. This interaction suggests that BT + CP in combination were synergistic and elicited a response greater than each would individually. A significant ($P < .01$) fat x protein x day interaction was found, however, indicating that the BT + CP diet did not consistently produce the highest intestinal cholesterogenesis on all days, but over the 48-day experimental period the diet produced the highest rates.

Little is known about the dietary regulation of cholesterogenesis

in the small bowel. The present study supports several laboratories (Carlson et al., 1978; Cayen, 1971; Chevallier and Lutton, 1973) which have reported a decreased cholesterogenesis in the small intestine when polyunsaturated fat was fed to rats. In all these studies, depressed intestinal cholesterol synthetic rates were attributed to increased intestinal cholesterol concentration, indicative of feedback control. In the present study, however, no increased intestinal cholesterol (Table 9) was observed in rats fed the polyunsaturated fat diets.

Protein effect The CP-fed rats had significantly greater ($P < .05$) mean small intestinal cholesterol synthetic rates than SP-fed rats, 0.70 and 0.49 μmoles/hour x g tissue, respectively. The CP-fed rats, however, had significantly higher intestinal cholesterogenesis with BT than with SO as the source of dietary fat. The implications of this finding have been discussed in the previous section.

No study was found in the literature that documented the effect of protein source on intestinal cholesterol biosynthesis. The forthcoming discussion, therefore, is only conjecture for the reason animal protein (CP) increased the rates of intestinal cholesterogenesis. Sugano et al. (1980) found the distribution of microsomal HMG-CoA reductase in the rat small intestine was modified by type of diet. Feeding a CP-containing diet, in comparison with an SP diet, may have resulted in a significant increase in the activity of the villous reductase. The increased activity of HMG-CoA reductase may have been potentiated when CP was fed in combination with BT.

The exact mechanism responsible for causing diet-induced modulations

in the distribution of reductase activity and cholesterogenesis is not known at this time. Several explanations are plausible. First, if a lowered absorption rate occurred in the ileum on a particular diet, this would produce a higher HMG-CoA reductase activity and increased cholesterogenesis (Sabine, 1977). Sugano et al. (1980) also found that HMG-CoA reductase and cholesterol synthesis is dependent upon the cholesterol to protein ratio in the villous cells. In addition, the diet-dependent difference in bile flow (Balmer and Zilversmit, 1974) and the distribution of bile salts along the small intestine (Eastwood and Boyd, 1967) may directly or indirectly account for changes in intestinal cholesterol synthetic rates. The CP could be affecting any one or all of these parameters to produce an increased intestinal cholesterogenesis.

Commercial rat diet The mean intestinal cholesterol synthetic rates in all rats on the experimental diets were less ($P < .001$) than the eight 0 time commercial diet-fed rats. Rats on the experimental diets were older than rats fed the commercial diet which could have contributed to the difference in intestinal cholesterogenesis.

Intestinal cholesterogenesis has been reported by some laboratories to be inhibited by dietary cholesterol (Bricker et al., 1972; Fishler-Mates et al., 1974). Thompson (1975) found cholesterol synthesis in the intestinal mucosa was 27% of the total production in control goats, but only 7% in cholesterol-fed goats. One possible explanation, therefore, for the lower intestinal cholesterol synthetic rates in rats fed experimental diets, is that the 0.2% dietary cholesterol inhibited cholesterogenesis. Another laboratory, however, did not observe any effect of

dietary cholesterol on intestinal cholesterol synthesis (Cayen, 1969).

The depression of cholesterol biosynthesis in the intestine by dietary cholesterol may be mediated through increased production of bile acids (Wilson, 1968). A high concentration of cholesterol in the intestinal lumen elicits greater bile acid production. In the present study, the dietary cholesterol may have increased bile acid production. It is possible that bile acids inhibit intestinal cholesterol synthesis either directly or indirectly by facilitating movement of some other inhibitory substance, such as cholesterol, across the epithelial cell membrane (Dietschy and Wilson, 1970a).

Sugano et al. (1980) demonstrated the HMG-CoA reductase activity in the ileum of rats fed a commercial diet was two times higher than that in the ileum of animals fed a purified diet. Nonnutritive fiber was postulated to be the dietary factor responsible for differences in intestinal HMG-CoA reductase activity and cholesterogenesis. The commercial diet allegedly contained 4% crude fiber (manufacturer's statement), the same percentage as that of cellulose in the purified diet. Sugano et al. (1980) found, however, that the commercial diet actually contained 30% dietary fiber. In the present experiment, a higher fiber content of the commercial rat diet, if actual, might account for the decreased plasma cholesterol (Table 7) and the increased intestinal cholesterol synthetic rate (Table 11) in the rats fed the commercial diet.

Terminal Body and Organ Weights

The rats initially weighed between 300 and 325 g. All rats grew well and were in good health until sacrificed. Mean body weights at necropsy for rats on the various experimental diets were statistically similar (Table 12). The rats fed SO, however, tended to be heavier at necropsy than rats fed BT, but the difference was not significant. Eight rats fed the commercial diet were sacrificed at the beginning of the 48-day experimental period, which resulted in lower average body weights. Rats fed the experimental diets gained an average of 85 g (2.1 g/day) over the 48 days (Table 13). The mean weight gains were not statistically different among the experimental diets.

Liver weights were expressed in grams and as a percentage of total body weight. No relationship between liver weights and dietary treatment was observed over time (Tables 12 and 13). Mean liver weights for all rats were statistically similar. Liver weights expressed as a percentage of body weight in rats fed the commercial diet were greater than rats fed the experimental diets because of a lowered total body weight.

Small intestine weights were also expressed in grams and as a percentage of total body weight. Mean small intestine weight and intestine weight as a percentage of body weight were greater ($P < .05$) in SP-fed rats than in CP-fed rats (Tables 12 and 13). Small intestine weight averaged 1 g higher in SP-fed rats. There was no relationship between small intestine weight and dietary fat source. Small intestine weights in rats fed the commercial diet and sacrificed at 0 days were greater than rats fed the experimental diets because of a lowered total body weight.

Table 12. Mean body and organ weights at necropsy

Diets	Parameters[a]				
	Body weight (g)	Liver weight (g)	Small intestine weight (g)	Liver as a % of body weight	Small intestine as a % of body weight
Commercial rat diet[b]	279 ± 20.58	12 ± 1.18	8 ± 0.47	4.4 ± 0.19	2.8 ± 0.07
SO + SP	371	12	7	3.2	1.9
SO + CP	364	12	6	3.3	1.8
BT + SP	339	11	7	3.2	2.0
BT + CP	348	11	6	3.2	1.8

Standard error of the treatment mean for body weight is ± 12.40.
Standard error of the treatment mean for liver weight (g) is ± 0.70.
Standard error of the treatment mean for small intestine weight (g) is ± 0.28.
Standard error of the treatment mean for liver as a % of body weight is ± 0.11.
Standard error of the treatment mean for small intestine as a % of body weight is ± 0.04.

[a]Diet means for rats sacrificed from 7 to 48 days (see also footnote b).

[b]Means are from eight rats fed a commercial rat diet and sacrificed at day 0.

Table 13. Body and organ weights at day of necropsy

Parameter	Day[a]											
	0[b]	7	9	11	13	15	18	22	30	36	42	48
Body weight (g)	279	319	348	340	331	347	359	354	372	387	384	404
Liver weight (g)	12	12	14	12	11	12	12	11	11	10	12	11
Small intestine weight (g)	8	7	7	7	7	7	6	6	6	7	7	7
Liver weight as a % of body weight	5	4	4	4	3	3	3	3	3	3	3	3
Small intestine as a % of body weight	3	2	2	2	2	2	2	2	2	2	2	2

Standard error of the time mean for body weight is ± 20.58.
Standard error of the time mean for liver weight (g) is ± 1.18.
Standard error of the time mean for small intestine weight (g) is ± 0.47.
Standard error of the time mean for liver as a % of body weight is ± 0.19.
Standard error of the time mean for small intestine as a % of body weight is ± 0.07.

[a] Day of necropsy.

[b] Means from eight rats fed a commercial diet and sacrificed at day 0.

Feed Consumption

Feed intake was controlled so that rats consumed an average of 10% (range 8-13%) of body weight per day thereby eliminating any effect of consumption on the cholesterol parameters measured. Sources of dietary fat and protein had no significant influence on total feed consumption during the 48-day experimental period (Table 14). Total feed consumption over the experimental period was significantly ($P < .0001$) influenced by day because the rats sacrificed later in the experimental period ate more total feed than rats sacrificed earlier (Table 15). The longer the rats consumed a given diet, the greater was the rats' total feed consumption. The average amount of feed consumed per day over the 48 days was statistically similar on all experimental diets (Table 14).

Feed consumption expressed per gram body weight was significantly greater ($P < .005$) for BT-fed rats than for SO-fed rats (Table 14). Animals fed BT consumed an average of 1.0 g feed per gram body weight while SO-fed rats consumed 0.9 g feed per gram body weight. The BT-fed rats were less efficient than the SO-fed rats because they required more feed for each gram of body weight. The BT diets may have been slightly more palatable than the SO diets causing the rats to have a higher intake per gram body weight on BT diets. Table 14 shows that BT-fed rats did have a higher total feed consumption even though it was not significant.

Table 14. Group mean feed consumptions

Diets	Parameters[a]		
	Total feed consumption (g)	Feed consumption/ g body weight (g)	Average g feed/day (g)
SO + SP	365	0.9	35
SO + CP	346	0.9	35
BT + SP	377	1.0	35
BT + CP	360	1.0	35

Standard error of the treatment mean for total feed consumption is ± 19.46.

Standard error of the treatment mean for feed consumption per gram body weight is ± 0.02.

Standard error of the treatment mean for average grams feed per day is ± 1.54.

[a] Diet means for rats sacrificed from 7 to 48 days.

Table 15. Feed consumptions

Parameter	Day[a] 7	9	11	13	15	18	22	30	36	42	48
Total feed consumption to day of sacrifice (g)	110	168	177	198	233	294	334	450	596	657	760
Feed consumption/g body weight to day of sacrifice (g)	0.3	0.5	0.5	0.6	0.7	0.8	0.9	1.2	1.5	1.7	1.9
Average feed/day to day of sacrifice (g)	37	42	35	33	33	37	34	32	34	33	33

Standard error of the day mean for total feed consumption is ± 32.26.
Standard error of the day mean for feed consumption per gram body weight is ± 0.03.
Standard error of the day mean for average grams feed per day is ± 2.56.

[a]Day of sacrifice.

SUMMARY

The experiment was conducted over a 48-day period and was designed to explore the influence of dietary fat and protein sources on tissue cholesterol parameters in the adult male rat. Experimental diets were isocaloric and consisted of combinations of fat, beef tallow (BT) or soybean oil (SO) and protein, casein protein (CP) or soy protein (SP), from plant or animal sources. Crystalline cholesterol (0.2%) was added to each experimental diet to assess its effect on tissue cholesterol levels and biosynthetic rates. Eight rats sacrificed at 0 time had consumed a commercial diet with no cholesterol supplementation. Feed intake was controlled so that rats consumed approximately 10% of body weight per day.

Relative rates of _in vitro_ cholesterol biosynthesis were evaluated using [1-^{14}C]-octanoic acid incorporated into DPS. Cholesterol synthetic rates in the liver and small intestine were expressed as μmoles of [1-^{14}C]-octanoic acid incorporated into DPS per gram wet weight of tissue per hour incubation. Radiolabeled octanoic acid was used as the cholesterol precursor in the present experiment because it has been shown to provide more accurate results than radiolabeled acetate (Dietschy and Brown, 1974; Dietschy and McGarry, 1974).

Serum cholesterol concentration was affected by dietary fat and protein sources. Animal sources of dietary fat (BT) and protein (CP) increased plasma cholesterol concentrations. Conversely, polyunsaturated fat (SO) and plant protein (SP) reduced plasma cholesterol concentrations.

Rats sacrificed at 0 time, for the most part, had lower plasma cholesterol levels than rats fed experimental diets. The addition of 0.2% cholesterol to the experimental diets may have been responsible for the higher plasma cholesterol concentrations. The differences may also be due to the age difference between rats fed the experimental diet and rats fed only the commercial diet. Rats appeared to adapt to cholesterol feeding over the 48-day period and probably have a sensitive control mechanism aimed at maintaining blood cholesterol concentrations regardless of dietary manipulations.

The study found that SO-fed rats had higher liver cholesterols than BT-fed rats regardless of dietary protein. Rats fed the CP diet had higher liver cholesterol levels compared to SP-fed rats. The mean liver cholesterol of rats on experimental diets and rats (0 time) on the commercial diet only were similar, indicating that dietary cholesterol probably had no effect on liver cholesterol levels.

The liver has an average cholesterol synthetic rate approximately equal to the small intestine. Reports on the influence of dietary fat and protein sources on hepatic cholesterogenesis have been conflicting. This study did not indicate that dietary polyunsaturated fat produced increased rates of cholesterol biosynthesis. On the contrary, there were no differences in hepatic cholesterogenesis between BT- and SO-fed rats. Other investigators, however, have reported increased turnover of cholesterol in animals fed polyunsaturated fat due to a combination of elevated biosynthetic and degradative rates of cholesterol. Results of the present study also showed no effect of protein source on liver cholesterol

synthetic rates. The study provided evidence that the hypocholesterolemic effect of plant protein in the rat is not related to decreased hepatic cholesterogenesis.

The small intestine plays a central role in overall cholesterol balance in the body. Cholesterol biosynthesis was determined in the terminal ileum. Several studies have demonstrated the caudal ileum to be the major site of intestinal cholesterogenesis. Dietary animal fat (BT) and protein (CP) were found to increase intestinal cholesterogenesis. The dietary combination of BT and CP produced twice the intestinal synthetic rate compared to other experimental diets causing a fat x protein interaction. The interaction suggested that BT and CP in combination were synergistic and elicited a response greater than each would individually. The intestinal synthetic rates in rats fed experimental diets containing cholesterol were less than rats fed only a commercial diet (sacrificed at 0 time), indicating a possible negative feedback inhibition by cholesterol on HMG-CoA reductase. There is support in the literature for this claim; however, the depression of intestinal cholesterogenesis by dietary cholesterol may be mediated through increased bile acid production.

No relationship between body or liver weight and dietary fat or protein source was observed during the experimental period. Intestinal weights, however, were greater in animals consuming SP. No relationship between small intestine weight and dietary fat source was observed.

Feed consumption was controlled so that rats consumed an average of 10% of body weight per day, thereby eliminating any effect of feed intake

on the cholesterol parameters measured. Sources of dietary fat and protein, therefore, had no influence on total feed consumption in rats.

More research is needed to increase our understanding of the effects of dietary fat and protein on cholesterol metabolism. Possible areas that should be examined in future research include:

1) a study to examine in greater depth the interaction between animal fat and protein with respect to intestinal cholesterogenesis;
2) an elucidation of other possible mechanisms (absorption, degradation, and excretion) responsible for changes in tissue cholesterol levels of animals fed different sources of fat and protein;
3) a study of the interactions of other dietary components with fat and protein insofar as cholesterol parameters are concerned;
4) an examination of other dietary constituents (carbohydrate, fiber, etc.) that may affect cholesterol metabolism; and
5) a study specifically designed to examine plasma cholesterol adaptation to dietary cholesterol over variable periods of time.

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ACKNOWLEDGMENTS

I wish to express my thanks and appreciation to Dr. Norman Jacobson for his friendship and guidance, and for his critical evaluation of this dissertation. I will never forget his support and encouragement and can never hope to repay his kindness. Thanks are extended to Dr. Jerry Young for his evaluation of this dissertation; to Dr. David Cox for his unselfish help and advice in the statistical analyses of the data. Many thanks are also extended to committee members Dr. A. Dare McGilliard and Dr. Donald D. Draper. Appreciation also is extended to Dr. Kenneth Wiggers for the grant money to conduct this research.

I am very grateful to Faith Bjork, Teresa Tovrea, Jeanne Stewart, and Marlene Richard for their laboratory assistance. I also wish to thank my fellow graduate students who provided answers to many of my questions.

I express my gratefulness and love to Ruth and Stacie for their understanding, encouragement, and devotion; and to my parents for their faithful support of my decisions.

This research was funded in part by a grant from the World Food Institute, Iowa State University.

APPENDIX

Table 16. Summary of data from day 0

Diet	Animal number	Plasma cholesterol concentration (mg/dl)	Tissue cholesterol (%)		Tissue cholesterol biosynthesis (μmoles/hr x g tissue)	
			Liver	Small intestine	Liver	Small intestine
Control	1	73	0.18	0.22	2.18	1.89
	2	70	0.13	0.22	1.18	2.74
	3	69	0.16	0.19	1.22	2.70
	4	59	0.26	0.19	2.30	3.27
	5	72	0.17	0.25	1.97	1.70
	6	50	0.28	0.25	0.65	0.14
	7	67	0.21	0.22	1.39	0.33
	8	64	0.27	0.24	2.69	1.00

Table 16 (Continued)

Diet	Animal number	Organ wet weight (g) Liver	Small intestine	Total body weight (g)	Organ weight as a % of body weight (%) Liver	Small intestine	Feed consumption (g) Total	Average/day
Control	1	14.35	7.00	286	5.02	2.45	--	--
	2	11.00	7.06	285	3.86	2.48	--	--
	3	14.24	9.24	278	5.12	3.32	--	--
	4	11.10	6.56	284	3.91	2.31	--	--
	5	12.30	7.91	263	4.68	3.01	--	--
	6	10.87	8.88	289	3.76	3.07	--	--
	7	12.68	7.92	285	4.45	2.78	--	--
	8	10.88	7.24	262	4.15	2.76	--	--

Table 17. Summary of data from day 7

Diet	Animal number	Plasma cholesterol concentration (mg/dl)	Tissue cholesterol (%)		Tissue cholesterol biosynthesis (μmoles/hr x g tissue)	
			Liver	Small intestine	Liver	Small intestine
SO + SP	1	111	0.43	0.15	2.40	0.02
	2	71	0.23	0.22	2.80	0.01
SO + CP	1	53	0.38	0.17	0.29	0.03
	2	77	0.31	0.29	1.98	0.03
BT + SP	1	65	0.18	0.30	0.79	0.39
	2	76	0.33	0.19	1.60	0.37
BT + CP	1	90	0.25	0.33	9.30	0.10
	2	99	0.46	0.21	0.95	0.51

Table 17 (Continued)

Diet	Animal number	Organ wet weight Liver (g)	Organ wet weight Small intestine (g)	Total body weight (g)	Organ weight as a % of body weight Liver (%)	Organ weight as a % of body weight Small intestine (%)	Feed consumption Total[a] (g)	Feed consumption Average/day (g)
SO + SP	1	12.13	8.10	329	3.69	2.46	97	32
	2	12.87	7.60	323	3.98	2.35	110	37
SO + CP	1	9.90	6.00	309	3.20	1.94	151	50
	2	11.73	6.07	319	3.68	1.90	100	33
BT + SP	1	12.33	6.73	317	3.89	2.12	98	33
	2	12.14	7.29	328	3.70	2.22	116	39
BT + CP	1	11.78	6.91	316	3.73	2.19	126	42
	2	15.00	7.53	312	4.81	2.41	84	28

[a]Feed consumed to day of sacrifice.

Table 18. Summary of data from day 9

Diet	Animal number	Plasma cholesterol concentration (mg/dl)	Tissue cholesterol (%)		Tissue cholesterol biosynthesis (μmoles/hr x g tissue)	
			Liver	Small intestine	Liver	Small intestine
SO + SP	1	76	0.22	0.23	1.17	0.86
	2	62	0.32	0.18	0.52	1.23
SO + CP	1	70	0.44	0.29	1.60	0.58
	2	98	0.23	0.27	0.75	0.41
BT + SP	1	86	0.20	0.24	0.85	0.43
	2	71	0.30	0.33	2.07	0.23
BT + CP	1	115	0.63	0.25	1.07	2.76
	2	113	0.24	0.11	2.56	1.56

Table 18 (Continued)

Diet	Animal number	Organ wet weight (g)		Total body weight (g)	Organ weight as a % of body weight (%)		Feed consumption (g)	
		Liver	Small intestine		Liver	Small intestine	Total[a]	Average/day
SO + SP	1	11.60	6.64	339	3.42	1.96	134	34
	2	12.74	7.55	347	3.67	2.18	184	46
SO + CP	1	12.09	6.00	343	3.52	1.75	150	38
	2	15.36	7.79	363	4.23	2.15	186	47
BT + SP	1	16.96	7.37	324	5.23	2.27	175	44
	2	12.02	5.68	346	3.47	1.64	145	36
BT + CP	1	14.43	8.59	363	3.98	2.37	180	45
	2	13.70	7.40	358	3.83	2.07	188	47

[a]Feed consumed to day of sacrifice.

Table 19. Summary of data from day 11

Diet	Animal number	Plasma cholesterol concentration (mg/dl)	Tissue cholesterol (%)		Tissue cholesterol biosynthesis (μmoles/hr x g tissue)	
			Liver	Small instestine	Liver	Small intestine
SO + SP	1	61	0.50	0.22	1.39	0.81
	2	73	0.37	0.30	1.22	0.28
SO + CP	1	42	0.42	0.22	1.06	0.38
	2	62	0.39	0.24	0.92	0.38
BT + SP	1	61	0.31	0.24	0.23	0.38
	2	90	0.28	0.26	0.26	0.26
BT + CP	1	66	0.27	0.24	0.47	0.53
	2	87	0.20	0.33	1.53	0.63

Table 19 (Continued)

Diet	Animal number	Organ wet weight (g)		Total body weight (g)	Organ weight as a % of body weight (%)		Feed consumption[a] (g)	
		Liver	Small intestine		Liver	Small intestine	Total	Average/day
SO + SP	1	12.25	6.42	341	3.59	1.88	171	34
	2	12.36	6.42	343	3.60	1.87	201	40
SO + CP	1	15.48	7.15	368	4.21	1.94	186	37
	2	13.66	7.89	369	3.70	2.14	197	39
BT + SP	1	11.57	5.86	321	3.60	1.83	168	34
	2	8.28	5.38	271	3.06	1.99	144	29
BT + CP	1	10.12	5.73	328	3.09	1.75	144	29
	2	14.52	7.49	376	3.86	1.99	205	41

[a]Feed consumed to day of sacrifice.

Table 20. Summary of data from day 13

Diet	Animal number	Plasma cholesterol concentration (mg/dl)	Tissue cholesterol		Tissue cholesterol biosynthesis	
			Liver	Small intestine	Liver	Small intestine
			(%)		(μmoles/hr x g tissue)	
SO + SP	1	64	0.70	0.24	0.59	1.18
	2	74	0.74	0.31	0.49	0.86
SO + CP	1	101	0.55	0.28	0.40	0.60
	2	104	0.82	0.37	0.70	0.64
BT + SP	1	71	0.40	0.24	0.55	0.33
	2	86	0.43	0.33	0.70	1.25
BT + CP	1	82	0.61	0.28	1.02	1.56
	2	91	0.52	0.32	1.63	2.19

Table 20 (Continued)

Diet	Animal number	Organ wet weight (g) Liver	Small intestine	Total body weight (g)	Organ weight as a % of body weight (%) Liver	Small intestine	Feed consumption (g) Total[a]	Average/day
SO + SP	1	10.77	7.42	345	3.12	2.15	152	25
	2	11.10	6.84	346	3.21	1.98	196	33
SO + CP	1	14.72	8.89	402	3.66	2.21	293	49
	2	9.56	6.35	334	2.86	1.90	201	34
BT + SP	1	13.53	9.10	362	3.74	2.51	228	38
	2	9.65	5.46	271	3.56	2.01	169	28
BT + CP	1	6.55	4.36	247	2.65	1.77	148	25
	2	13.42	7.57	343	3.91	2.21	197	33

[a]Feed consumed to day of sacrifice.

Table 21. Summary of data from day 15

Diet	Animal number	Plasma cholesterol concentration (mg/dl)	Tissue cholesterol (%)		Tissue cholesterol biosynthesis (μmoles/hr x g tissue)	
			Liver	Small intestine	Liver	Small intestine
SO + SP	1	74	0.46	0.29	0.66	0.62
	2	80	0.39	0.25	1.08	1.08
SO + CP	1	66	0.49	0.23	0.34	0.10
	2	85	0.32	0.24	0.89	0.79
BT + SP	1	93	0.22	0.35	0.70	0.39
	2	70	0.48	0.25	1.84	0.69
BT + CP	1	95	0.57	0.36	0.40	1.20
	2	87	0.34	0.27	0.77	0.83

Table 21 (Continued)

Die	Animal number	Organ wet weight (g) Liver	Small intestine	Total body weight (g)	Organ weight as a % of body weight (%) Liver	Small intestine	Feed consumption Total[a]	Average/day (g)
SO + SP	1	8.00	5.05	261	3.07	1.93	169	24
	2	14.26	7.44	383	3.72	1.94	254	36
SO + CP	1	12.15	6.24	380	3.20	1.64	248	35
	2	13.29	7.50	347	3.83	2.16	199	28
BT + SP	1	13.55	8.07	381	3.56	2.12	294	42
	2	10.00	5.49	312	3.21	1.76	243	35
BT + CP	1	12.31	6.54	362	3.40	1.81	213	30
	2	12.44	6.28	347	3.59	1.81	241	34

[a]Feed consumed to day of sacrifice.

Table 22. Summary of data from day 18

Diet	Animal number	Plasma cholesterol concentration (mg/dl)	Tissue cholesterol		Tissue cholesterol biosynthesis	
			Liver	Small intestine	Liver	Small intestine
			(%)		(μmoles/hr x g tissue)	
SO + SP	1	78	0.45	0.40	0.22	0.49
	2	91	0.40	0.38	0.89	0.36
SO + CP	1	87	0.34	0.37	0.27	0.13
	2	94	0.54	0.40	0.15	0.48
BT + SP	1	101	0.29	0.33	0.12	0.76
	2	101	0.30	0.37	4.24	0.26
BT + CP	1	126	0.25	0.45	0.52	1.61
	2	84	0.30	0.31	0.64	1.61

Table 22 (Continued)

Diet	Animal Number	Organ wet weight (g) Liver	Small intestine	Total body weight (g)	Organ weight as a % of body weight (%) Liver	Small intestine	Feed consumption Total[a]	Average/day (g)
SO + SP	1	9.88	5.51	353	2.80	1.56	291	36
	2	15.33	8.56	417	3.68	2.05	358	45
SO + CP	1	15.26	6.34	392	3.89	1.62	311	39
	2	10.67	5.03	336	3.18	1.50	229	29
BT + SP	1	8.72	5.39	297	2.94	1.81	222	28
	2	10.66	7.11	340	3.14	2.09	320	40
BT + CP	1	11.51	6.11	340	3.39	1.80	287	36
	2	11.67	7.33	395	2.95	1.86	334	42

[a]Feed consumed to day of sacrifice.

Table 23. Summary of data from day 22

Diet	Animal number	Plasma cholesterol concentration (mg/dl)	Tissue cholesterol		Tissue cholesterol biosynthesis	
			Liver	Small intestine	Liver	Small intestine
			(%)		(μmoles/hr x g tissue)	
SO + SP	1	89	0.29	0.24	1.28	0.11
	2	89	0.57	0.28	0.59	0.14
SO + CP	1	127	0.53	0.26	0.26	0.26
	2	119	0.31	0.26	0.30	0.68
BT + SP	1	105	0.36	0.20	0.26	0.28
	2	84	0.23	0.28	1.02	0.87
BT + CP	1	96	0.46	0.32	0.05	1.04
	2	88	0.27	0.31	0.12	0.27

Table 23 (Continued)

Diet	Animal number	Organ wet weight Liver (g)	Organ wet weight Small intestine (g)	Total body weight (g)	Organ weight as a % of body weight Liver (%)	Organ weight as a % of body weight Small intestine (%)	Feed consumption Total[a]	Feed consumption Average/day (g)
SO + SP	1	12.20	7.73	377	3.24	2.05	322	32
	2	13.76	6.76	437	3.15	1.55	412	41
SO + CP	1	9.64	5.58	330	2.92	1.69	270	27
	2	14.35	7.34	400	3.59	1.83	428	43
BT + SP	1	7.83	5.64	305	2.57	1.85	357	36
	2	9.97	7.02	333	2.99	2.11	238	24
BT + CP	1	12.31	6.53	404	3.05	1.62	417	42
	2	5.96	4.10	250	2.38	1.64	232	23

[a]Feed consumed to day of sacrifice.

Table 24. Summary of data from day 30

Diet	Animal number	Plasma cholesterol concentration (mg/dl)	Tissue cholesterol (%)		Tissue cholesterol biosynthesis (μmoles/hr x g tissue)	
			Liver	Small intestine	Liver	Small intestine
SO + SP	1	67	0.52	0.54	0.31	0.31
	2	115	0.47	0.34	1.28	0.32
SO + CP	1	97	1.58	0.41	0.32	0.23
	2	92	0.98	0.44	0.47	0.50
BT + SP	1	92	0.31	0.34	0.44	0.17
	2	86	0.25	0.36	0.67	1.17
BT + CP	1	105	0.42	0.40	0.74	0.29
	2	115	0.67	0.50	1.36	1.17

Table 24 (Continued)

Diet	Animal number	Organ wet weight (g) Liver	Small intestine	Total body weight (g)	Organ weight as a % of body weight (%) Liver	Small intestine	Feed consumption Total[a] (g)	Average/day (g)
SO + SP	1	7.37	4.92	305	2.42	1.61	384	27
	2	14.40	7.64	405	3.56	1.89	438	31
SO + CP	1	15.94	6.71	445	3.58	1.51	565	40
	2	8.53	5.23	356	2.40	1.47	408	29
BT + SP	1	12.28	8.00	436	2.82	1.83	578	41
	2	11.31	7.04	347	3.26	2.03	346	25
BT + CP	1	13.23	6.75	380	3.48	1.78	534	38
	2	6.74	5.03	300	2.25	1.68	381	27

[a]Feed consumed to day of sacrifice.

Table 25. Summary of data from day 36

Diet	Animal number	Plasma cholesterol concentration (mg/dl)	Tissue cholesterol		Tissue cholesterol biosynthesis	
			Liver	Small intestine	Liver	Small intestine
			(%)		(μmoles/hr x g tissue)	
SO + SP	1	83	1.12	0.35	0.42	0.41
	2	59	0.70	0.31	0.38	0.25
SO + CP	1	77	1.42	0.40	0.65	0.21
	2	58	0.60	0.27	1.43	1.41
BT + SP	1	70	0.42	0.31	0.60	0.43
	2	44	0.35	0.34	0.25	0.67
BT + CP	1	75	0.38	0.36	0.67	0.24
	2	71	0.28	0.33	1.30	1.42

Table 25 (Continued)

Diet	Animal number	Organ wet weight (g) Liver	Small intestine	Total body weight (g)	Organ weight as a % of body weight (%) Liver	Small intestine	Feed consumption Total[a]	Average/day (g)
SO + SP	1	14.71	9.46	484	3.04	1.95	695	41
	2	10.07	6.38	413	2.44	1.54	595	35
SO + CP	1	7.00	4.06	294	2.38	1.38	447	26
	2	9.66	6.11	394	2.45	1.55	617	36
BT + SP	1	12.71	9.05	421	3.02	2.15	665	39
	2	9.09	7.10	367	2.48	1.93	626	37
BT + CP	1	8.07	5.21	327	2.47	1.59	514	30
	2	12.53	6.79	400	3.13	1.70	613	36

[a]Feed consumed to day of sacrifice.

Table 26. Summary of data from day 42

Diet	Animal number	Plasma cholesterol concentration (mg/dl)	Tissue cholesterol (%)		Tissue cholesterol biosynthesis (μmoles/hr x g tissue)	
			Liver	Small intestine	Liver	Small intestine
SO + SP	1	43	0.51	0.26	1.10	0.15
	2	59	1.03	0.26	0.21	0.12
SO + CP	1	87	0.77	0.26	0.70	0.48
	2	57	0.60	0.30	0.28	0.25
BT + SP	1	60	0.23	0.22	0.14	0.20
	2	77	0.32	0.23	1.44	0.41
BT + CP	1	129	0.29	0.27	0.19	0.62
	2	68	0.30	0.25	1.65	1.64

Table 26 (Continued)

Diet	Animal number	Organ wet weight (g) Liver	Small intestine	Total body weight (g)	Organ weight as a % of body weight (%) Liver	Small intestine	Feed consumption Total[a]	Average/day (g)
SO + SP	1	9.16	6.78	359	2.55	1.89	634	32
	2	14.96	7.75	424	3.53	1.83	757	38
SO + CP	1	16.30	8.19	478	3.41	1.71	767	38
	2	7.94	4.44	306	2.59	1.45	418	21
BT + SP	1	6.27	5.53	294	2.13	1.88	538	27
	2	14.89	8.27	492	3.03	1.68	845	42
BT + CP	1	17.03	6.80	437	3.90	1.56	784	39
	2	6.34	5.17	284	2.23	1.82	515	26

[a]Feed consumed to day of sacrifice.

Table 27. Summary of data from day 48

Diet	Animal number	Plasma cholesterol Concentration (mg/dl)	Tissue cholesterol (%)		Tissue cholesterol biosynthesis (μmoles/hr x g tissue)	
			Liver	Small intestine	Liver	Small intestine
SO + SP	1	54	0.45	0.29	0.59	0.61
	2	51	0.37	0.34	0.11	0.05
SO + CP	1	59	0.90	0.26	0.45	0.07
	2	66	0.34	0.36	0.19	0.07
BT + SP	1	59	0.32	0.38	0.74	0.76
	2	76	0.45	0.30	0.07	0.37
BT + CP	1	58	0.42	0.47	0.24	0.26
	2	79	0.33	0.39	0.22	0.04

Table 27 (Continued)

Diet	Animal number	Organ wet weight Liver (g)	Organ wet weight Small intestine (g)	Total body weight (g)	Organ weight as a % of body weight Liver (%)	Organ weight as a % of body weight Small intestine (%)	Feed consumption Total[a] (g)	Feed consumption Average/day (g)
SO + SP	1	10.17	7.08	387	2.63	1.83	663	29
	2	11.90	7.50	435	2.74	1.72	803	35
SO + CP	1	16.97	7.42	470	3.61	1.58	788	34
	2	6.19	4.29	262	2.36	1.64	464	20
BT + SP	1	12.86	8.28	471	2.73	1.76	900	39
	2	10.95	7.18	423	2.59	1.70	888	39
BT + CP	1	8.15	5.69	362	2.25	1.57	772	34
	2	11.23	7.06	425	2.64	1.66	802	35

[a]Feed consumed to day of sacrifice.